A P P L I C A T I O N

for

UNITED STATES LETTERS PATENT

on

MOLECULES THAT HOME TO VARIOUS SELECTED ORGANS OR TISSUES

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DATE OF DEPOSIT: June 27, 2003

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Sheets of Drawings: Four (4)

Docket No.: 66821-209 (P-LJ 5778)

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MOLECULES THAT HOME TO VARIOUS SELECTED ORGANS OR TISSUES

This invention was made with government support under grant numbers CA 74238 and CA 30199 awarded by the National Institutes of Health.

BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

The present invention relates generally to the fields of molecular medicine and drug delivery and, more specifically, to molecules that home to a specific organ or tissue.

BACKGROUND INFORMATION

Although the effect of a particular pathology often is manifest throughout the body of the afflicted person, generally, the underlying pathology may affect only a single organ or tissue. It is rare, however, that a drug or other treatment will target only the diseased organ or tissue. More commonly, treatment results in undesirable side effects due, for example, to generalized toxic effects throughout the patient's body. It would be desirable to selectively target organs or tissues, for example, for treatment of diseases associated with the target organ or tissue. In particular, targeting of an organ or tissue can be useful for directing the expression of a gene to a certain organ or tissue because incorporation of a foreign gene into nontargeted cells can cause unwanted side effects such as malignant transformation.

Most therapeutic substances are delivered to the target organ or tissue through the circulation. The endothelium, which lines the internal surfaces of blood vessels, is the first cell type encountered by a circulating therapeutic substance in the target organ or tissue. These cells provide a target for selectively directing therapies to an organ or tissue.

Endothelium can have distinct morphologies and biochemical markers in different tissues. The blood vessels of the lymphatic system, for example, express various adhesion proteins that serve to guide lymphocyte homing. For example, endothelial cells present in lymph nodes express a cell surface marker that is a ligand for L-selectin and endothelial cells in Peyer's patch venules express a ligand for the $\alpha_4\beta_7$ integrin. These ligands are involved in specific lymphocyte homing to their respective lymphoid organs. Thus, linking a drug to L-selectin or to the $\alpha_4\beta_7$ integrin may provide a means for targeting the drug to diseased lymph nodes or Peyer's patches, respectively, provided that these molecules do not bind to similar ligands present in a significant number of other organs or tissues.

Although the homing molecules present in the blood vessels of non-lymphoid tissues have not been clearly defined, certain observations of lymphocyte circulation suggest that organ and tissue specific endothelial markers exist. Similarly, the homing or metastasis of particular types of tumor cells to specific organs or tissues further suggests that organ and tissue specific markers may exist. Thus, a need exists to identify molecules that can bind to such organ or tissue specific markers and, therefore, can

home to the organ or tissue. The present invention satisfies this need and provides related advantages as well.

SUMMARY OF THE INVENTION

The present invention provides molecules that selectively home to various normal organs or tissues, including to lung, skin, pancreas, retina, prostate, ovary, lymph node, adrenal gland, liver or gut. For example, the invention provides lung homing peptides such as those containing a GFE motif, including the peptides CGFECVRQCPERC (SEQ ID NO: 1) and CGFELETC (SEQ ID NO: 2); skin homing peptides such as CVALCREACGEGC (SEQ ID NO: 3); pancreas homing peptides such as the peptide SWCEPGWCR (SEQ ID NO: 4); and retina homing peptides such as those containing an RDV motif, including the peptides CSCFRDVCC (SEQ ID NO: 5) and CRDVVSVIC (SEQ ID NO: 6).

The invention also provides conjugates, comprising an organ or tissue homing molecule linked to a moiety. Such a moiety can be a therapeutic agent such as a toxin, an agent that inhibits cell death, an agent that alters the production or activity of a deleterious or beneficial substance by a cell, or an agent that alters proliferation of a cell exposed to the agent. A moiety also can be a detectable agent such as a radionuclide, or a tag such as a chambered microdevice or an insoluble chromatography support. Such conjugates of the invention are useful for directing the moiety to a selected organ or tissue.

The invention also provides methods of using an organ homing molecule of the invention to diagnose

or treat a pathology of the lung, skin, pancreas, retina, prostate, ovary, lymph node, adrenal gland, liver or gut by administering a molecule that homes to the selected organ or tissue to a subject having or suspected of having a pathology. For example, a pathology of lung, skin, pancreas, retina, prostate, ovary, lymph node, adrenal gland, liver or gut can be treated by administering to a subject having the pathology a conjugate comprising an appropriate organ homing molecule linked to a therapeutic agent. Similarly, a method of identifying a selected organ or tissue or diagnosing a pathology in a selected organ by administering to a subject a conjugate comprising an appropriate organ homing molecule linked to a detectable agent.

The invention further provides methods of identifying a target molecule in lung, pancreas, skin, retina, prostate, ovary, lymph node, adrenal gland, liver or gut by detecting selective binding of the target molecule to a lung, skin, pancreas, retina, prostate, ovary, lymph node, adrenal gland, liver or gut homing molecule, respectively. For example, a peptide that selectively homes to lung can be attached to a solid matrix, a sample of lung can be obtained and passed over the affinity matrix under conditions that allow specific binding of the target molecule, and the target molecule can be collected and identified. Thus, the invention also provides a target molecule, which binds an organ homing molecule, particularly a lung, skin, pancreas, retina, prostate, ovary, lymph node, adrenal gland, liver or gut homing molecule. Such a target molecule can be useful, for example, for raising an antibody specific for the target molecule.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the results of three rounds of *in vivo* panning of a CX_6C (SEQ ID NO: 26) library for identifying molecules that home to lung. Phage recovered from the lung five minutes after injection of 10^{10} transducing units into the tail vein of mice were amplified and reinjected in two consecutive rounds. The number of phage recovered per gram of lung, kidney or brain is indicated for each round, with bars representing standard error of the mean from triplicate platings.

Figures 2A to 2D show the selectivity of phage displaying a lung (Figures 2A and 2B), skin (Figure 2C) or pancreas (Figure 2D) homing peptides. Selected phage expressing peptides that home to lung (Figure 2A, CGFECVRQCPERC, SEQ ID NO: 1, "GFE-1"; Figure 2B, CGFELETC, SEQ ID NO: 2, "GFE-2"); skin (Figure 2C, CVALCREACGEGC, SEQ ID NO: 3) or pancreas (Figure 2D, SWCEPGWCR, SEQ ID NO: 4) were individually amplified, injected into mice and recovered (Example I. The amount of phage displaying a selected peptide that was recovered per gram of lung, skin or pancreas or control kidney or brain was determined. The amount of unselected (control) phage recovered from lung, skin, pancreas, kidney or brain also was determined. Bars indicate standard error of the mean from triplicate platings.

Figures 3A and 3B show the effect of coadministration of GST-fusion proteins on the homing of phage displaying lung or skin homing peptides.

In Figure 3A, 100 µg or 500 µg of GST-CGFECVRQCPERC (SEQ ID NO: 1, "GFE-1") or 500 µg GST was coinjected into mice with 10^9 transducing units of the individually amplified phage displaying the lung homing sequence CGFECVRQCPERC (SEQ ID NO: 1). The recovery of phage after 5 minutes of circulation from lung and kidney was determined, with bars indicating standard error of the mean from triplicate platings.

In Figure 3B, 10^9 transducing units of individually amplified phage displaying either the lung homing peptides CGFECVRQCPERC (SEQ ID NO: 1, "GFE-1") or CGFELETC (SEQ ID NO: 2, "GFE-2") or the skin homing peptide CVALCREACGEGC (SEQ ID NO: 3) were coinjected into mice with 500 µg of the cognate GST-fusion peptide. Control mice (not shown) were injected with the selected phage and 500 µg of GST. The percentage of inhibition of selected phage homing to lung or skin in the presence of the cognate GST-fusion peptide compared to GST is shown.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides organ and tissue homing molecules and methods of using these molecules to target a moiety to a selected organ or tissue. The molecules of the invention, which were identified essentially by the method of *in vivo* panning (U.S. Patent No. 5,622,699, issued April 22, 1997, which is incorporated herein by reference), include peptides that home to various normal organs or tissues, including lung, skin, pancreas, retina, prostate, ovary, lymph node, adrenal gland, liver or gut, and to organs bearing tumors, including to lung bearing lung tumors and to pancreas bearing a pancreatic tumor. For

example, the invention provides lung homing peptides, including the peptides CGFECVRQCPERC (SEQ ID NO: 1) and CGFELETC (SEQ ID NO: 2), each of which contains a tripeptide GFE motif, and the peptide GIGEVEVC (SEQ ID NO: 8). The invention also provides skin homing peptides such as the peptide CVALCREACGEGC (SEQ ID NO: 3); pancreas homing peptides such as the peptide SWCEPGWCR (SEQ ID NO: 4) and retina homing peptides such as the peptides CSCFRDVCC (SEQ ID NO: 5) and CRDVVSVIC (SEQ ID NO: 6), each of which contains a tripeptide RDV motif. Examples of peptides that home to prostate, ovary, lymph node, adrenal gland, liver and gut are also provided (see Tables 2 to 11). It should be recognized that motifs common to particular organ homing peptides can be identified by simple inspection of the peptides. For example, inspection of Table 9 reveals that the peptides AGCSVTVCG (SEQ ID NO: 319) and AGCVQSQCY (SEQ ID NO: 370) share an AGC motif; the peptides LECRRWCRCD (SEQ ID NO: 328) and LECVANLCT (SEQ ID NO: 337) share an LEC motif; and the peptides SECAYRACS (SEQ ID NO: 319) and SECYTGSCP (SEQ ID NO: 375) share an SEC motif. In addition, several of these peptides were isolated more than one time (see asterisks in Table 9), indicating that such motifs are relevant to the ability of the peptides to selectively home. Peptides comprising the particular motifs disclosed herein, as well as other motifs identifiable by inspection of the disclosed peptides, are considered within the claimed invention, provided that the motif is not an RGD motif.

The homing molecules of the invention are useful for targeting a moiety to a particular organ or tissue. Thus, the invention provides conjugates, comprising an organ homing molecule linked to a moiety.

Such moieties can be a therapeutic agent such as a virus; a viral gene therapy vector; a drug; a detectable or imaging agent such as a radionuclide; or a tag such as biotin. As disclosed herein, such organ homing molecules of the invention, particularly conjugates of the invention, can be used to detect or visualize a selected organ or tissue or to diagnose or treat a pathology in a selected organ or tissue. An organ homing molecule of the invention also can be used to isolate the target molecule that is expressed in the selected organ or tissue and binds the organ homing molecule. For convenience, a molecule of the invention that homes to a selected organ or tissue is referred to as an "organ homing molecule."

As used herein, the term "molecule" is used broadly to mean an organic compound having at least one reactive group that can be varied by substituting one or more different groups. An organic molecule can be a drug; a nucleic acid molecule, including RNA or DNA; a peptide; a variant or modified peptide or a peptide mimetic; a protein or a fragment thereof; an oligosaccharide; a lipid; a glycolipid; or a lipoprotein.

An organic molecule can be a naturally occurring molecule, which can be a product of nature in that the groups comprising the organic molecule and the bonds linking the groups are produced by biological processes. For example, a naturally occurring organic molecule can be an RNA molecule or a fragment thereof, which can be isolated from a cell or expressed from a recombinant nucleic acid molecule. Similarly, a peptide is considered a naturally occurring organic molecule, even if it is produced by chemical synthesis,

since the amino acid groups and bonds linking the groups can be produced by normal biological processes and the peptide, itself, can be produced in a cell due, for example, to proteolytic degradation of a protein containing the peptide.

An organic molecule also can be a nonnaturally occurring molecule. Such molecules have chemical groups or bonds that are not normally produced by biological processes. For example, a nucleic acid sequence containing nonnaturally occurring nucleoside analogs or phosphorothioate bonds that link the nucleotides and protect against degradation by nucleases are examples of nonnaturally occurring molecules. A ribonucleotide containing a 2-methyl group, instead of the normal hydroxyl group, bonded to the 2'-carbon atom of ribose residues, is an example of a non-naturally occurring RNA molecule that is resistant to enzymatic and chemical degradation. Other examples of nonnaturally occurring organic molecules include RNA containing 2'-aminopyrimidines, such RNA being 1000x more stable in human serum and urine as compared to naturally occurring RNA (see Lin et al., Nucl. Acids Res., 22:5229-5234 (1994); and Jellinek et al., Biochemistry, 34:11363-11372 (1995), each of which is incorporated herein by reference).

For convenience, the term "peptide" is used broadly herein to mean peptides, polypeptides, proteins and fragments of proteins. Other molecules useful in the invention include peptoids, peptidomimetics and the like. With respect to the organ or tissue homing peptides of the invention, peptidomimetics, which include chemically modified peptides, peptide-like molecules containing nonnaturally occurring amino

acids, peptoids and the like, have the binding activity of an organ homing peptide upon which the peptidomimetic is derived (see, for example, "Burger's Medicinal Chemistry and Drug Discovery" 5th ed., vols. 1 to 3 (ed. M.E. Wolff; Wiley Interscience 1995), which is incorporated herein by reference). Peptidomimetics provide various advantages over a peptide, including that a peptidomimetic can be stable when administered to a subject, for example, during passage through the digestive tract and, therefore, useful for oral administration.

Methods for identifying a peptidomimetic are well known in the art and include, for example, the screening of databases that contain libraries of potential peptidomimetics. For example, the Cambridge Structural Database contains a collection of greater than 300,000 compounds that have known crystal structures (Allen et al., Acta Crystallogr. Section B, 35:2331 (1979)). This structural depository is continually updated as new crystal structures are determined and can be screened for compounds having suitable shapes, for example, the same shape as an organ or tissue homing molecule, as well as potential geometrical and chemical complementarity to a target molecule bound by an organ or tissue homing peptide. Where no crystal structure of a homing peptide or a target molecule, which binds an organ or tissue homing molecule, is available, a structure can be generated using, for example, the program CONCORD (Rusinko et al., J. Chem. Inf. Comput. Sci. 29:251 (1989)). Another database, the Available Chemicals Directory (Molecular Design Limited, Informations Systems; San Leandro CA), contains about 100,000 compounds that are commercially available and also can be searched to

identify potential peptidomimetics of an organ or tissue homing molecule.

The term "nucleic acid molecule" also is used broadly to mean any polymer of two or more nucleotides, which are linked by a covalent bond such as a phosphodiester bond, a thioester bond, or any of various other bonds known in the art as useful and effective for linking nucleotides. Such nucleic acid molecules can be linear, circular or supercoiled, and can be single stranded or double stranded DNA or RNA or can be a DNA/RNA hybrid.

As used herein, the term "library" means a collection of molecules. A library can contain a few or a large number of different molecules, varying from about two to about 10^{15} molecules or more. The chemical structure of the molecules of a library can be related to each other or be diverse. If desired, the molecules constituting the library can be linked to a common or unique tag, which can facilitate recovery and/or identification of the molecule.

Methods for preparing libraries containing diverse populations of various types of molecules such as peptides, peptoids and peptidomimetics are well known in the art and various libraries are commercially available (see, for example, Ecker and Crooke, Biotechnology 13:351-360 (1995), and Blondelle et al., Trends Anal. Chem. 14:83-92 (1995), and the references cited therein, each of which is incorporated herein by reference; see, also, Goodman and Ro, Peptidomimetics for Drug Design, in "Burger's Medicinal Chemistry and Drug Discovery" Vol. 1 (ed. M.E. Wolff; John Wiley & Sons 1995), pages 803-861, and Gordon et al., J. Med.

Chem. 37:1385-1401 (1994), each of which is incorporated herein by reference). Where a molecule is a peptide, protein or fragment thereof, the molecule can be produced *in vitro* directly or can be expressed from a nucleic acid, which can be produced *in vitro*. Methods of synthetic peptide and nucleic acid chemistry are well known in the art.

A library of peptide molecules also can be produced, for example, by constructing a cDNA expression library from mRNA collected from a cell, tissue, organ or organism of interest. Methods for producing such libraries are well known in the art (see, for example, Sambrook et al., Molecular Cloning: A laboratory manual (Cold Spring Harbor Laboratory Press 1989), which is incorporated herein by reference). Preferably, a peptide encoded by the cDNA is expressed on the surface of a cell or a virus containing the cDNA. For example, cDNA can be cloned into a phage vector such as fuse 5 (Example I), wherein, upon expression, the encoded peptide is expressed as a fusion protein on the surface of the phage.

In addition, a library of molecules can be a library of nucleic acid molecules, which can be DNA, RNA or analogs thereof. For example, a cDNA library can be constructed from mRNA collected from a cell, tissue, organ or organism of interest, or by collecting genomic DNA, which can be treated to produce appropriately sized fragments using restriction endonucleases or methods that randomly fragment genomic DNA. A library comprising RNA molecules also can be constructed by collecting RNA from cells or by synthesizing the RNA molecules chemically. Diverse

libraries of nucleic acid molecules can be made using solid phase synthesis, which facilitates the production of randomized regions in the molecules. If desired, the randomization can be biased to produce a library of nucleic acid molecules containing particular percentages of one or more nucleotides at a position in the molecule (U.S. Patent No.: 5,270,163, issued December 14, 1993, which is incorporated herein by reference).

If desired, the nucleic acid molecules can be nucleic acid analogs that are less susceptible to degradation by nucleases. For example, RNA molecules containing 2'-0-methylpurine substitutions on the ribose residues and short phosphorothioate caps at the 3'- and 5'-ends exhibit enhanced resistance to nucleases (Green et al., Chem. Biol., 2:683-695 (1995), which is incorporated herein by reference). Similarly, RNA containing 2'-amino- 2'-deoxypyrimidines or 2'-fluro- 2'-deoxypyrimidines is less susceptible to nuclease activity (Pagratis et al., Nature Biotechnol., 15:68-73 (1997), which is incorporated herein by reference). Furthermore, L-RNA, which is a stereoisomer of naturally occurring D-RNA, is resistant to nuclease activity (Nolte et al., Nature Biotechnol., 14:1116-1119 (1996); Klobmann et al., Nature Biotechnol., 14:1112-1115 (1996); each of which is incorporated herein by reference). Such RNA molecules and methods of producing them are well known and routine (see Eaton and Piekern, Ann. Rev. Biochem., 64:837-863 (1995), which is incorporated herein by reference). DNA molecules containing phosphorothioate linked oligodeoxynucleotides are nuclease resistant (Reed et al., Cancer Res. 50:6565-6570 (1990), which is incorporated herein by reference). Phosphorothioate-3'

hydroxypropylamine modification of the phosphodiester bond also reduces the susceptibility of a DNA molecule to nuclease degradation (see Tam et al., Nucl. Acids Res., 22:977-986 (1994), which is incorporated herein by reference). If desired, the diversity of a DNA library can be enhanced by replacing thymidine with 5-(1-pentynyl)-2'-deoxoridine (Latham et al., Nucl. Acids Res. 22:2817-2822 (1994), which is incorporated herein by reference). Such modified nucleic acid molecules can be useful for the manufacture of a library or for the purpose of being a tag, which is described later below.

As disclosed herein, *in vivo* panning for the purpose of identifying an organ or tissue homing molecule comprises administering a library to a subject, collecting an organ or tissue sample and identifying an organ or tissue homing molecule using various methods well known in the art. Generally, the presence of an organ or tissue homing molecule in a collected organ or tissue is identified based on one or more characteristics common to the molecules present in the library, then the structure of a particular organ or tissue homing molecule can be determined.

A highly sensitive detection method such as mass spectrometry (MS), either alone or in combination with a method such as gas chromatography (GC), can be used to identify homing molecules that are closely related even when present in small amounts in a selected organ or tissue. For example, GC in combination with MS was used to identify two major and four minor lidocaine metabolites following lidocaine injection into rats and the analysis of urine (Coutts et al., J. Chromotogr. 421:267-280 (1987), which is

incorporated herein by reference). Similarly, where a library comprises diverse molecules based generally on the structure of an organic molecule such as a drug, an organ or tissue homing molecule can be identified by determining the presence of a parent peak for the particular molecule.

If desired, the selected organ or tissue can be processed using a method such as HPLC, which can be used to obtain an enriched fraction of molecules having a defined range of molecular weights or polarity or the like from a complex mixture. The enriched fraction of molecules then can be further analyzed for the purposes of identifying organ or tissue homing molecules. For example, HPLC coupled with GC and MS were used to identify seven metabolites of a vitamin D analogue after injection of dihydrotachysterol 3 into a rat and fractionation of an isolated perfused kidney (Porteous et al., Biomed. Environ. Mass Spectrum 16:87-92 (1988), which is incorporated herein by reference). Conditions for HPLC will depend on the structure of the particular molecule and can be optimized by those skilled in the art based on knowledge of the molecule.

The organ homing molecules present in a collected sample of organ or tissue can be recovered from the sample by incubation in a solution having a defined salt concentration and temperature. Selective extraction also can be used to obtain different fractions of organic molecules by sequentially incubating a collected sample in one or more solutions. Such solutions can have a different salt concentration or can effect extraction of an organic homing molecule at a particular temperature. The resulting eluates from the collected sample can be collected separately

or can be pooled into one or more fractions and the organ homing molecules can be detected and identified. Similarly, methods for bulk removal of potentially interfering cellular materials such as DNA, RNA, proteins, lipids or carbohydrates are well known in the art. Such methods can be used to enrich for the particular organ homing molecule from potentially contaminating materials in the collected sample and to increase the sensitivity of detecting the molecule.

Ease of identification of an organ or tissue homing molecule, particularly an untagged molecule, depends upon various factors, including the presence of potentially contaminating background cellular material. For example, where the homing molecule is an untagged peptide, a larger number must home to the organ or tissue in order to identify the specific peptides over the background of cellular protein. In contrast, a much smaller amount of an untagged homing molecule such as a drug is identifiable because such molecules normally are generally absent from or present in very small numbers in the body. In this situation, a highly sensitive method such as MS can be used to identify an organ homing molecule. The skilled artisan will recognize that the method of identifying a molecule will depend, in part, on the structure of the particular molecule.

As disclosed herein, a sufficient number of molecules selectively home to a selected organ or tissue during *in vivo* panning such that the molecules readily can be identified. For example, peptides that were identified two or more times in a particular collected organ (see Table 1). For example, of forty clones sequenced from various selected organs, the gut

homing peptide YSGKWGK (SEQ ID NO: 9) was present in 22% of the clones; the ovary homing peptides EVRSRLS (SEQ ID NO: 10) and RVGLVAR (SEQ ID NO: 11) each was present in 22% of the clones; and the liver homing peptide VKSVCRT (SEQ ID NO: 12) was present in 11% of the clones (see Table 1). Similarly, the lung homing peptides CLAKENVVC (SEQ ID NO: 13) and CGFECVRQCPERC (SEQ ID NO: 1); the skin homing peptide CVALCREACGEGC (SEQ ID NO: 3); and the retina homing peptide CGEFKVGC (SEQ ID NO: 14) each was independently isolated several times during *in vivo* panning of the respective organs, as were other organ homing peptides (see Tables 2 to 11; peptides marked with asterisk). These results demonstrate that a substantial fraction of the identified organ homing molecules have the same structure or, in many cases, share conserved motifs.

Following various *in vivo* panning screens, hundreds of thousands to millions of phage expressing homing peptides were recovered from the respective organ or tissue. Generally, the phage collected from a round of *in vivo* panning were plated on agar, about 250 to 300 clones were selected, grown in 5 ml cultures, then pooled and readministered for a subsequent round of *in vivo* panning ("regular method"). However, in some experiments, 1000 clones were selected, grown in 2 ml cultures, then pooled and administered for a subsequent round of screening; or the entire agar plate was scraped and all of the phage were cultured together and administered for a subsequent round of screening. The peptide inserts of various isolated phage were determined such that, of the millions of phage that homed, only a small number of sequences were identified. These results indicate that specific types of homing molecules can be present in relatively large

proportions in an organ or tissue following *in vivo* homing, thereby increasing the ease with which the molecules can be identified.

Where an organ or tissue homing molecule is a nucleic acid molecule, various assay methods can be used to substantially isolate or identify the molecule. For example, PCR can be particularly useful for identifying the presence of the homing molecule because, in principle, PCR can detect the presence of a single nucleic acid molecule (see, for example, Erlich, PCR Technology: Principles and Applications for DNA Amplification (Stockton Press (1989), which is incorporated herein by reference). PCR also has been used to amplify nucleic acid molecules that bind to a predetermined target *in vitro* and, when the nucleic acids were rendered resistant to nucleases and administered to a subject, they modulated biological processes such as lymphocyte trafficking *in vivo* (see, for example, Hicke et al., J. Clin. Invest. 98:2688-2692 (1996), which is incorporated herein by reference). These findings indicate that nucleic acid molecules are sufficiently stable when administered into the circulation of a subject such that *in vivo* panning can be used to identify nucleic acid molecules that selectively home to an organ or tissue *in vivo*.

The molecules of a library can be tagged, which can facilitate recovery or identification of the organ homing molecules. As used herein, the term "tag" means a physical, chemical or biological moiety such as a plastic or metallic microbead, an oligonucleotide or a bacteriophage, respectively, that is linked to a molecule of the library. Methods for tagging a molecule are well known in the art (Hermanson,

Bioconjugate Techniques, (Academic Press 1996), which is incorporated herein by reference). The link between a molecule and a tag can be a covalent or a non-covalent bond and, if desired, the link can be selectively cleavable from the molecule.

As used herein, the term "shared tag" means a physical, chemical or biological moiety that is common to each molecule in a library. A shared tag can be used to identify the presence of a molecule of the library in a sample or to substantially isolate the molecules from a sample following *in vivo* panning. For example, a library that comprises a population of diverse molecules such as nucleic acids can be linked to a shared tag. If the shared tag is biotin, for example, a nucleic acid homing molecule can be substantially isolated from a selected organ or tissue by binding, for example, to a streptavidin affinity column. The presence of the organ or tissue homing nucleic acid molecule also can be detected by binding with a labeled streptavidin. A peptide such as the hemagglutinin antigen also can be a shared tag, which, when linked to each molecule in a library, allows the use of an antibody specific for the hemagglutinin antigen to substantially isolate homing molecules from a selected organ or tissue. Furthermore, a molecule or a support containing a molecule can be linked to a hapten such as 4-ethoxy-methylene-2-phenyl-2-oxazoline-5-one (phOx), which can be bound by an anti-phOx antibody linked to a magnetic bead as a means to recover the homing molecule. Methods for purifying phOx labeled conjugates are known in the art and the materials for performing these procedures are commercially available (Invitrogen, La Jolla CA; Promega Corp., Madison WI).

A shared tag also can be a nucleic acid sequence that can be used to identify the presence of molecules of the library in a sample or to substantially isolate molecules of a library from a sample. For example, each of the molecules of a library can be linked to the same selected nucleotide sequence, which constitutes the shared tag. An affinity column containing a nucleotide sequence that is complementary to the shared tag then can be used to isolate the homing molecules from an organ or tissue sample by hybridizing to the shared tag linked to the molecules. A nucleotide sequence complementary to a portion of the shared tag also can be used as a PCR primer such that the presence of molecules containing the shared tag can be identified in a sample by PCR.

A tag also can be a specific or a unique tag. As used herein, the term "specific tag" means a physical, chemical or biological tag that is linked to a molecule in a library and that is unique for the particular molecule. A specific tag is particularly useful if it is readily identifiable. A nucleotide sequence that is unique for a particular molecule of a library is an example of a specific tag, for example, a unique oligonucleotide tag linked to each peptide of a library or peptides (see, for example, Brenner and Lerner, Proc. Natl. Acad. Sci., *USA* 89:5381-5383 (1992), which is incorporated herein by reference). Upon homing to an organ or tissue, the homing peptide can be identified by determining the sequence of the unique oligonucleotide tag using, for example, PCR (see, for example, Erlich, PCR Technology: Principles and Applications for DNA Amplification (Stockton Press 1989), which is incorporated herein by reference). Similarly, the nucleic acid sequence encoding a peptide

displayed on a phage is another example of a specific nucleic acid tag, since sequencing of the nucleic acid identifies the amino acid sequence of the expressed peptide (see Example I). Such unique oligonucleotide sequence tags, when linked to other libraries of molecules, can be used to identify the sequence of the homing molecule linked thereto.

A shared tag and specific tag, in combination, can be particularly useful for isolating and identifying an organ or tissue homing molecule when the homing molecule is present in minute quantities. For example, each molecule of a library can be linked to an oligonucleotide tag which contains two portions; an internal unique nucleotide sequence tag and shared flanking 5' and 3' nucleotide tags that serve as primer binding sites for use in PCR. Each molecule, therefore, contains an oligonucleotide tag having a unique portion to identify the homing molecule and a shared portion to provide PCR primer binding sites. Such a tagged molecule, upon homing to a selected organ or tissue, can be identified by performing PCR using primers that hybridize to the shared flanking 5' and 3' nucleotide tags, then performing DNA sequencing to determine the nucleotide sequence of the internal unique sequence tag. The PCR product can be sequenced directly using one of the PCR primers or the PCR product can be cloned into a vector and the DNA sequence determined by routine methods well known in the art.

Various other combinations of shared and unique tags can be used. For example, each of the molecules in a library can be linked to a specific nucleotide sequence tag (see, for example, Brenner and

Lerner, *supra*, 1992), which also contains a shared 3' nucleotide sequence that can be a primer binding site for use in PCR, and can be further linked to a shared tag such as biotin. Upon homing to an organ or tissue, the particular homing molecule can be substantially isolated from an organ or tissue sample based on the biotin tag. The isolated molecules can then be identified, for example, by PCR based DNA sequencing of the specific tag using the shared 3' nucleotide sequence of the nucleotide tag as a primer binding site.

A tag also can serve as a support. As used herein, the term "support" means a tag having a defined surface to which a molecule can be attached. In general, a tag useful as a support is a shared tag. For example, a support can be a biological tag such as a virus or virus-like particle such as a bacteriophage ("phage"); a bacterium such as *E. coli*; or a eukaryotic cell such as a yeast, insect or mammalian cell; or can be a physical tag such as a liposome or a microbead, which can be composed of a plastic, agarose, gelatin or other biological or artificial material. If desired, a shared tag useful as a support can have linked thereto a specific tag.

As exemplified herein, a peptide suspected of being able to home to a selected normal organ or tissue such as lung, skin, pancreas, retina, prostate, ovary, lymph node, adrenal gland, liver or gut, or to an organ or tissue containing a tumor, for example, a lung containing lung tumors or a pancreas containing a pancreatic tumor, was expressed as the N-terminus of a fusion protein, wherein the C-terminus consisted of a phage coat protein (see Example I). Upon expression of

the fusion protein, the C-terminal coat protein linked the fusion protein to the surface of a phage such that the N-terminal peptide was in a position to interact with a target molecule in the organ or tissue. Thus, a molecule having a shared tag was formed by the linking of a peptide to a phage, wherein the phage provided a biological support, the peptide molecule was linked as a fusion protein, the phage-encoded portion of the fusion protein acted as a spacer molecule, and the nucleic acid encoding the peptide provided a specific tag allowing identification of organ and tissue homing peptides.

Where a molecule is linked to a support, the tagged molecule comprises the molecule attached to the surface of the support, such that the part of the molecule suspected of being able to interact with a target molecule in a cell in the subject is positioned so as to be able to participate in the interaction. For example, where the homing molecule is suspected of being a ligand for a growth factor receptor, the binding portion of the molecule attached to a support is positioned so it can interact with the growth factor receptor on a cell in an organ or tissue. If desired, an appropriate spacer can be positioned between the molecule and the support such that the ability of the potential organ or tissue homing molecule to interact with the target molecule is not hindered. A spacer molecule also can contain a reactive group, which provides a convenient and efficient means of linking a molecule to a support and, if desired, can contain a tag, which can facilitate recovery or identification of the molecule (see Hermanson, *supra*, 1996).

In general, a support should have a diameter less than about 10 µm to about 50 µm in its shortest dimension, such that the support can pass relatively unhindered through the capillary beds present in the subject so as to not occlude circulation. In addition, a support can be biologically inert, so that it does not perturb the normal expression of cell surface molecules or normal physiology of the subject. In addition, a support can be excretable or biodegradable, particularly where the subject used for *in vivo* panning is not sacrificed to collect a sample of a selected organ or tissue.

As used herein, the term "*in vivo* panning," when used in reference to the identification of an organ or tissue homing molecule, means a method of screening a library by administering the library to a subject and identifying a molecule that selectively homes to an organ or tissue in the subject (U.S. Patent No. 5,622,699, *supra*, 1997). The term "administering to a subject", when used in referring to a library of molecules or a portion of such a library, is used in its broadest sense to mean that the library is delivered to a selected organ or tissue in the subject, which, generally, is a vertebrate, particularly a mammal such as a human. Libraries of molecules can be administered by any route or means of administration, such as intravenously, intramuscularly, orally, optically, ocularly, intraperitoneally, nasally, vaginally, rectally, into the uterus, into a chamber of the eye, into the central or peripheral nervous system, by inhalation, by topical administration, or by injection into any normal organ or tissue or into a pathological region such as a tumor or an organ or

tissue involved in a pathology, particularly into the circulatory system of the organ or tissue.

A library can be administered to a subject, for example, by injecting the library into the circulation of the subject such that the molecules pass through the selected organ or tissue; after an appropriate period of time, circulation is terminated, for example, by perfusion through the heart or by removing a sample of the organ or tissue (Example I; U.S. Patent No. 5,622,699, *supra*, 1997; see, also, Pasqualini and Ruoslahti, Nature 380:364-366 (1996), which is incorporated herein by reference). Alternatively, a cannula can be inserted into a blood vessel in the subject, such that the library is administered by perfusion for an appropriate period of time, after which the library can be removed from the circulation through the cannula or the subject can be sacrificed or anesthetized to collect an organ or tissue sample. A library also can be shunted through one or a few organs or tissues including a selected organ or tissue, by cannulation of the appropriate blood vessels in the subject. It is recognized that a library also can be administered to an isolated perfused organ or tissue. Such panning in an isolated perfused organ or tissue can be useful to identify molecules that bind to the organ or tissue.

The use of *in vivo* panning to identify organ or tissue homing molecules is exemplified herein by screening a phage peptide display library in mice and identifying peptides that selectively homed to lung, pancreas, skin and others, and in rats, for peptides that homed to retina (Examples I and II). However, phage libraries that display other protein molecules,

including, for example, an antibody or an antigen binding fragment of an antibody such an Fv, Fd or Fab fragment; a hormone receptor such as a growth factor receptor; or a cell adhesion receptor such as an integrin or a selectin also can be used to practice the invention. Variants of such molecules can be constructed using well known methods such as random, site directed or codon based mutagenesis (see Huse, U.S. Patent No. 5,264,563, issued November 23, 1993, which is incorporated herein by reference) and, if desired, peptides can be chemically modified, for example, by introducing a disulfide bridge, following expression of the phage but prior to administration to the subject. Thus, many different types of phage display libraries can be screened by *in vivo* panning.

Phage display technology provides a means for expressing a diverse population of random or selectively randomized peptides. Various methods of phage display and methods for producing diverse populations of peptides are well known in the art. For example, Ladner et al. (U.S. Patent No. 5,223,409, issued June 29, 1993, which is incorporated herein by reference) describe methods for preparing diverse populations of binding domains on the surface of a phage. In particular, Ladner et al. describe phage vectors useful for producing a phage display library, as well as methods for selecting potential binding domains and producing randomly or selectively mutated binding domains.

Similarly, Smith and Scott (Meth. Enzymol. 217:228-257 (1993); see, also, Scott and Smith, Science 249: 386-390 (1990), each of which is incorporated herein by reference) describe methods of producing

phage peptide display libraries, including vectors and methods of diversifying the population of peptides that are expressed (see, also, Huse, WO 91/07141 and WO 91/07149, each of which is incorporated herein by reference; see, also, Example I). Phage display technology can be particularly powerful when used, for example, with a codon based mutagenesis method, which can be used to produce random peptides or randomly or desirably biased peptides (Huse, U.S. Patent No. 5,264,563, *supra*, 1993). These or other well known methods can be used to produce a phage display library, which can be subjected to the *in vivo* panning method of the invention in order to identify a peptide that homes to a selected organ or tissue.

In addition to screening a phage display library, *in vivo* panning can be used to screen various other types of libraries. For example, nucleic acid molecules that bind to a cell surface receptor have been described (see O'Connell et al., Proc. Natl. Acad. Sci., *USA* 93:5883-5887 (1996); Tuerk and Gold, Science 249:505-510 (1990); Gold et al., *supra* (1995), each of which is incorporated herein by reference). These *in vitro* results indicate that a library of nucleic acid molecules also can be examined by *in vivo* panning to identify nucleic acid molecules that home to a selected organ or tissue. Additional libraries suitable for screening include, for example, oligosaccharide libraries (York et al., Carb. Res. 285:99-128, (1996); Liang et al., Science 274:1520-1522, (1996); and Ding et al., Adv. Expt. Med. Biol. 376:261-269, (1995), each of which is incorporated by reference); lipoprotein libraries (de Kruif et al., FEBS Lett. 399:232-236, (1996), which is incorporated herein by reference); glycoprotein or

glycolipid libraries (Karaoglu et al., J. Cell Biol. 130:567-577 (1995), which is incorporated herein by reference); or chemical libraries containing, for example, drugs or other pharmaceutical agents (Gordon et al., J. Med. Chem. 37:1385-1401 (1994); Ecker and Crook, Bio/Technology 13:351-360 (1995); each of which is incorporated by reference). Such libraries, if desired, can be tagged, which can facilitate recovery of the molecule from an organ or tissue or its identification as previously described.

In vivo panning provides a method for directly identifying molecules that can selectively home to an organ or tissue. As used herein, the term "home" or "selectively home" means that a particular molecule binds relatively specifically to a target molecule present in the organ or tissue, particularly in the vasculature present in the organ or tissue, following administration to a subject. In general, selective homing is characterized, in part, by detecting at least a two-fold (2x) greater selective binding of the molecule to an organ or tissue as compared to a control organ or tissue.

Selective homing of a molecule to a selected organ or tissue can be due to selective recognition by the molecule of a particular cell target molecule such as a cell surface protein present on a cell in the organ or tissue. Selectivity of homing is dependent on the particular target molecule being expressed on only one or a few different cell types, such that the molecule homes to only one or a few organs or tissues. In this regard, most different cell types, particularly cell types that are unique to an organ or tissue, can express unique target molecules. Thus, in organs such

as liver, spleen or lymph node, where blood circulates through sinusoids formed by the cells specific for the organ, *in vivo* panning can be useful for identifying molecules that home to the particular organ or tissue.

It should be recognized that, in some instances, a molecule can localize nonspecifically to an organ or tissue. For example, *in vivo* panning of a phage display library can result in high background in organs such as liver and spleen, which contain a marked component of the reticuloendothelial system (RES). Thus, nonspecific binding of molecules due to uptake by the RES of such an organ or tissue can make identifying an organ or tissue homing molecule more difficult. However, as disclosed herein, potential nonspecific binding can be circumvented, for example, by perfusion through the heart prior to collecting the selected organ or tissue (Example I).

In addition, selective homing readily can be distinguished from nonspecific binding by detecting differences in the abilities of different individual phage to home to an organ or tissue. For example, selective homing can be identified by combining a putative homing molecule such as a peptide expressed on a phage with an excess of non-infective phage or with about a five-fold excess of phage expressing unselected peptides, injecting the mixture into a subject and collecting a sample of the organ or tissue. In the latter case, for example, provided the portion of injected phage in which an organ or tissue homing peptide is sufficiently low so as to be nonsaturating for the target molecule, a determination that greater than about 20% of the phage in the organ or tissue contain the putative homing molecule is demonstrative

evidence that the peptide expressed by the phage is a selective organ or tissue homing molecule. In addition, nonspecific localization can be distinguished from selective homing by performing competition experiments using, for example, phage expressing a putative organ or tissue homing peptide in combination with an excess amount of the "free" peptide (see Example II).

Various methods can be used to prevent nonspecific localization of a molecule to organs or tissues, such as those containing a component of the RES. For example, as disclosed herein, perfusion of a solution through the heart shortly after initiating phage circulation decreased the background binding and allowed identification of peptides that selectively home to lung and liver, both of which contain a component of the RES (see Example II). Furthermore, coadministration of nonreplicating control phage with a phage display library reduced nonspecific phage trapping in organs such as liver and spleen, which also contain a component of the RES. This approach allowed identification of molecules that selectively home to liver (Example II). Thus, a library of molecules attached to a support can be coadministered with an excess of the support to a subject to inhibit nonspecific binding in an organ or tissue.

Nonspecific uptake by a component of the RES also can be prevented by administering a blocking agent that inhibits uptake by the RES. For example, polystyrene latex particles or dextran sulfate can be administered to the subject prior to the administration of the library (see Kalin et al., Nucl. Med. Biol. 20:171-174 (1993); Illum et al., J. Pharm. Sci.

75:16-22 (1986); Takeya et al., J. Gen. Microbiol. 100:373-379 (1977), each of which is incorporated herein by reference). Such pre-administration of dextran sulfate 500 or polystyrene microspheres has been used to block nonspecific uptake of a test substance by Kupffer cells, which are the RES component of the liver (Illum et al., *supra*, 1986). Similarly, nonspecific uptake of agents by the RES has been blocked using carbon particles or silica (Takeya et al., *supra*, 1977) or a gelatine colloid (Kalin et al., *supra*, 1993). Thus, many methods useful for inhibiting nonspecific uptake by the RES are known in the art and routinely used.

Methods of decreasing nonspecific phage trapping include using phage that display a low background binding to a particular organ or tissue. For example, Merrill et al. (Proc. Natl. Acad. Sci., *USA* 93:3188-3192 (1996), which is incorporated herein by reference) selected lambda-type phage that are not taken up by the RES and, as a result, remain in the circulation for a prolonged period of time. A comparable filamentous phage variant, for example, can be selected using similar methods.

Selective homing can be demonstrated by determining if a homing molecule for a selected organ or tissue is relatively specific. For example, the amount of homing molecule in a selected organ or tissue can be compared to a control or different organ or tissue. Selective homing also can be demonstrated by showing that molecules that home to an organ or tissue, as identified by one round of *in vivo* panning, are enriched for in a subsequent round of *in vivo* panning. For example, phage expressing the peptides

CGFECVRQCPERC (SEQ ID NO: 1) and CGFELETC (SEQ ID NO: 2) were enriched for in the second and third rounds of *in vivo* panning from lung and exhibited a 35-fold and 9-fold enrichment, respectively, as compared to unselected phage (see Example II.B). Furthermore, no selective homing to kidney or brain was detected.

As used herein, the term "selected organ or tissue" is used in its broadest sense to mean a normal organ or tissue or an organ or tissue having a pathology, for example, lung containing lung tumors, to which a molecule can selectively home. Thus, the term "organ or tissue" is used broadly to mean any tissue or organ including a normal or pathological cell type such as a cancer cell, in which case the selected organ or tissue can be a primary tumor or a metastatic lesion.

In general, a selected organ or tissue contains a cell, which can be a cell of the vasculature, that expresses a particular target molecule such as a cell surface protein to which a homing molecule can bind. By performing at least two rounds of *in vivo* panning, the selectivity of homing of the molecule for the selected organ or tissue can be determined. As discussed below, however, in some cases a homing molecule can home to more than one selected organ or tissue, in which case the molecule is considered to be able to selectively home to a family of selected organs or tissues. Generally, however, molecules that home to more than one or a few different organs or tissue are not particularly useful since an advantage of the homing molecules of the invention is that they allow targeting of a particular organ or tissue.

The term "control organ or tissue" is used to mean an organ or tissue other than the selected organ or tissue. A control organ or tissue is characterized by the inability of the organ or tissue homing molecule to home to the control organ or tissue and, therefore, is useful for identifying selective binding of a molecule to a selected organ or tissue (Example II). Where an organ or tissue homing molecule is identified based on its ability to home to a pathologic lesion in an organ or tissue, the control organ or tissue can be a corresponding portion of the selected organ or tissue that does not exhibit the pathologic lesion.

A control organ or tissue can be collected, for example, to identify nonspecific binding of the molecule or to determine the selectivity of homing of the molecule. In addition, nonspecific binding can be identified by administering, for example, a control molecule, which is known not to home to an organ or tissue but is chemically similar to a putative homing molecule. Alternatively, where the administered molecules are linked to a support, administration of the support, alone, can be used to identify nonspecific binding. For example, a phage that does not contain a peptide fusion protein can be administered to a subject and the selected organ or tissue can be examined to determine the level of nonspecific binding of the phage support.

The steps of administering the library to the subject, collecting a selected organ or tissue and identifying the molecules that home to the organ or tissue, comprise a single round of *in vivo* panning. Although not required, one or more additional rounds of *in vivo* panning generally are performed. Where an

additional round of *in vivo* panning is performed, the molecules recovered from the selected organ or tissue in the previous round are administered to a subject, which can be the same subject used in the previous round, where only a part of the organ or tissue was collected.

By performing a second round of *in vivo* panning, the relative binding selectivity of the molecules recovered from the first round can be determined by administering the identified molecules to a subject, collecting the selected organ or tissue, and determining whether more phage displaying a particular molecule are recovered from the organ or tissue following the second round of screening as compared to those recovered following the first round. Although not required, a control organ or tissue also can be collected and the molecules recovered from the selected organ or tissue can be compared with those recovered from the control organ or tissue. Ideally, few if any molecules are recovered from a control organ or tissue following a second or subsequent round of *in vivo* panning. Generally, however, a proportion of the molecules also will be present in a control organ or tissue. In this case, the ratio of molecules in the selected organ or tissue as compared to the control organ or tissue (selected:control) can be determined. Additional rounds of *in vivo* panning can be used to determine whether a particular molecule homes only to the selected organ or tissue or can recognize a target expressed in one or more other organs or tissues that is identical or is sufficiently similar to the target in the originally selected organ or tissue.

In general, a library of molecules, which contains a diverse population of random or selectively randomized molecules of interest, is prepared, then administered to a subject. Some time after administration, the selected organ or tissue is collected and the molecules present in the selected organ or tissue are identified (see Example I). If desired, one or more control organs or tissues or a part of a control organ or tissue are sampled as well. For example, mice injected with a phage peptide display library, after about 1 to 5 minutes, were anesthetized, then snap frozen or perfused through the heart to terminate circulation of the phage. Lung, pancreas or other organs or tissues and one or more control organs were collected and the phage present in the selected and control organs were collected. The peptides that selectively homed to the respective organs or tissues were identified (Example II and Tables 1 to 11).

As exemplified herein, experimental animals were sacrificed to collect the selected or control organ or tissue. It should be recognized, however, that only a part of an organ or tissue need be collected to recover a molecule that homes to that organ or tissue. Similarly, only part of an organ or tissue need be collected as a control. Thus, for example, following administration of a library of molecules to a subject, a part of the selected organ or tissue can be collected by biopsy, the homing molecules can be collected and, if desired, amplified and readministered to the same subject for a second round of *in vivo* panning. Where the molecule that is to be administered a second time to the same subject is tagged or linked, for example, to a support, the tag or support should be biologically inert and biodegradable

or excretable, so as not to interfere with subsequent rounds of screening.

In vitro screening of phage libraries previously was used to identify peptides that bind to antibodies or to cell surface receptors (Smith and Scott, *supra*, 1993). For example, *in vitro* screening of phage peptide display libraries identified novel peptides that specifically bound to integrin adhesion receptors (Koivunen et al., J. Cell Biol. 124:373-380 (1994a), which is incorporated herein by reference) and to the human urokinase receptor (Goodson et al., Proc. Natl. Acad. Sci., *USA* 91:7129-7133 (1994), which is incorporated herein by reference). Similarly, *in vitro* screening of nucleic acid molecules identified molecules that specifically bind to antibodies, cell surface receptors or organic molecules (Gold et al., *supra*, 1993, 1995, 1997). For example, RNA molecules that specifically bind to HIV-1 reverse transcriptase were identified using purified HIV-1 reverse transcriptase as the target molecule (Green et al., J. Mol. Biol., 247:60-68 (1995), which is incorporated herein by reference). These *in vitro* methods were performed using defined, well-characterized target molecules in an artificial system. However, such *in vitro* studies provide no insight as to whether a molecule that binds *in vitro* also can bind to the target *in vivo*. For example, endothelial cells grown in culture tend to lose their tissue-specific differences (Pauli and Lee, Lab. Invest. 58:379-387 (1988), which is incorporated herein by reference). Thus, a molecule that binds to a target on a cell *in vitro* may not bind *in vivo* because the target may not be present on the cell. Furthermore, such *in vitro*

methods are limited in that they require prior knowledge of the target molecule and yield little if any information regarding *in vivo* utility. For example, Goodson et al. (*supra*, 1994) utilized cultured cells to express a recombinant urokinase receptor to obtain binding peptides. However, the urokinase receptor is expressed in cells of many different organs and tissues and, therefore, a molecule that binds to it can interact with many organs or tissues and would not be considered an organ or tissue homing molecule within the present invention.

In contrast to *in vitro* panning methods, *in vivo* panning requires no prior knowledge or the availability of a known target molecule to identify a molecule that binds to a target molecule that is expressed *in vivo*. Also, since "nontargeted" organs or tissues are present during the screening, the probability of isolating organ or tissue homing molecules that lack selectivity of homing is greatly reduced. Furthermore, in obtaining organ or tissue homing molecules by *in vivo* panning, any molecules that may be particularly susceptible to degradation in the circulation *in vivo* due, for example, to a metabolic activity, will be selected against and will not be recovered. Thus, *in vivo* panning provides significant advantages over previous methods by identifying molecules that selectively home *in vivo* and, if desired, the target molecule present on a selected organ or tissue.

The identification of the organ homing molecules that selectively home to various normal tissues and to pathologic lesions in a particular organ or tissue, as exemplified herein, indicates that

particular endothelial cell target molecules expressed the selected organ or tissue reflects the physiologic or pathologic state of the organ or tissue. Such organ homing molecules that selectively home to an organ or tissue based on a particular physiologic or pathologic condition occurring in the organ or tissue can be identified using the *in vivo* panning method and the selectivity of the homing molecules for the pathologic or physiologic condition of the organ or tissue can be confirmed by immunohistological analysis (Example III). For example, molecules that home to pancreas afflicted with pancreatitis can be identified by *in vivo* panning of a subject having pancreatitis and selectively of the homing molecule can be confirmed by using immunohistochemistry to compare homing of the molecule in normal pancreas with homing in a pancreas afflicted with pancreatitis.

Homing molecules selective for a normal organ or tissue or an organ or tissue exhibiting a pathological state can be useful for detecting the presence or absence of the pathology. For example, following administration of a prostate homing molecule conjugated to an imaging moiety to a subject, the prostate can be visualized. If the image is abnormal, for example, if the size of the prostate is other than that expected for a size and age matched subject, the imaging result can indicate an abnormal physiologic condition or pathologic condition afflicting the prostate. For example, a conjugate comprising an imaging agent and a prostate homing molecule that homes to normal, but not to abnormal prostate, can be administered to a subject. The identification, for example, of a region of the prostate that does not bind the homing molecule can indicate the occurrence of

abnormal blood flow in the prostate and can be diagnostic of a pathologic condition such as the presence of a prostate tumor. A conjugate comprising a molecule that homes to prostate tumor tissue, but not to normal prostate, can be used to image a prostate tumor directly.

A homing molecule selective for an organ or tissue can be used to deliver a therapeutic agent to the organ or tissue. Such selective targeting of the agent can increase the effective amount of the agent delivered to the target organ or tissue, while reducing the likelihood the agent will have an adverse effect on other organs or tissues. For example, a lung homing molecule can be used to deliver, to the lung of a cystic fibrosis patient, a gene encoding the cystic fibrosis transmembrane receptor (CFTR), which is defective in cystic fibrosis. Thus, the organ homing molecules of the invention are particularly useful for *in vivo* gene therapy, since they provide a means to direct a gene to a desired target organ, thereby increasing the likelihood that the target cells will receive the gene and decreasing the likelihood that normal, nontarget, cells will be adversely affected. A lung homing molecule also can be used to direct a therapeutic agent to the lung, thus sparing nontarget organs or tissues from the toxic effects of the agent. For example, in alveolar bacterial pneumonia, a lung homing molecule can be useful for directing an antibiotic to the afflicted region of the lung, thus increasing the effective amount of the drug at the desired site.

Due to the conserved nature of cellular receptors and of ligands that bind a particular

receptor, the skilled artisan would recognize that an organ or tissue homing molecule identified using *in vivo* panning in a mouse or rat also can bind to the corresponding target molecule in the selected organ or tissue of a human or other mammalian species. Such a homing molecule identified using an experimental animal readily can be examined for the ability to bind to the corresponding organ or tissue in a human subject by demonstrating, for example, that the molecule also can bind selectively *in vitro* to a sample of the selected organ or tissue obtained from a human subject. Alternatively, primary cells or established cell lines derived from a human organ or tissue can be used to test for the *in vitro* binding of the homing molecule. Similarly, primary cells or established cell lines that reflect a particular human organ or tissue pathology can be used to test the binding of homing molecules selective for the pathology. Animal models such as primate models of human pathologies are known and also can be used to test for the homing of the molecules using *in vivo* panning. Thus, routine methods can be used to confirm that an organ or tissue homing molecule identified using *in vivo* panning in an experimental animal also can bind an organ or tissue-specific target molecule in a human subject. Furthermore, *in vitro* contacting of a homing molecule with a sample suspected of containing a selected organ, tissue or pathology can identify the presence of the selected organ, tissue or pathology in the sample. Having identified the target molecule by *in vivo* panning, the artisan would know that it is the true target for an organ homing molecule and, therefore, would know that the target molecule could be used *in vitro* to identify addtional organ homing molecules that likely would be specific for the target molecule *in vivo*. Such potential organ homing

molecules then could be examined by *in vivo* panning to confirm organ homing ability.

In vivo panning was used to identify peptides expressed by phage that selectively homed to lung, skin, pancreas, retina, prostate, ovary, lymph node, adrenal gland, liver or gut, and to lung containing lung tumors or pancreas containing a pancreatic tumor (Examples II and IV; see, also, Tables 2 to 11). Due to the large size of the phage (300 nm) and the short time the phage were allowed to circulate, it is unlikely that a substantial number of phage would have exited the circulatory system. Indeed, immunohistochemical studies of various organ and tissue homing molecules demonstrated that the molecules primarily home to and bind endothelial cell surface markers of the vasculature. Thus, the invention provides molecules such as peptides that selectively home to the vasculature of a selected organ or tissue.

Phage peptide display libraries were constructed essentially as described by Smith and Scott (*supra*, 1993; see, also, Koivunen et al., Biotechnology 13:265-270 (1995); Koivunen et al., Meth. Enzymol. 245:346-369 (1994b), each of which is incorporated herein by reference). In some libraries, at least one codon encoding cysteine also was included in each oligonucleotide so that cyclic peptides could be formed through disulfide linkages (Example I). Upon performing *in vivo* panning, peptides that selectively home to lung, pancreas, skin, retina, prostate, ovary, lymph node, adrenal gland, liver or gut or to lung containing lung tumors or to pancreas containing a pancreatic tumor were obtained. Thus, the invention

provides various organ homing molecules that selectively home to particular organs or tissue.

Remarkably, some organ homing peptides independently were recovered up to four or more times during a round of the *in vivo* panning procedure (see, for example, Table 1). In addition, various peptides that homed to particular organs or tissues shared conserved amino acid sequence motifs. For example, some lung homing peptides shared a GFE motif; some retina homing peptides shared a RDV motif; and some adrenal gland homing peptides shared a LPR motif (see Tables 2, 6 and 11, respectively). Since it is known, for example, that the tripeptide RGD motif is sufficient for integrin binding (Ruoslahti, Ann. Rev. Cell Devel. Biol. 12:697 (1996); Koivunen et al., *supra*, 1995; WO 95/14714), the results disclosed herein indicate that many ligand/receptor interactions can derive their specificity from recognition motifs as small as tripeptides.

None of the sequences of the disclosed organ homing peptides exhibited significant similarity with known ligands for endothelial cell receptors. While many of the organ homing peptides may be contained within larger peptides or proteins, it is not known whether they are able to impart a homing function onto the larger molecule. Based on the previous finding that RGD mediates integrin binding when present within larger peptides and proteins, one skilled in the art would recognize, however, that such homing peptides and motifs could impart a homing function when located within a larger peptide or protein. However, such naturally occurring endogenous peptides and proteins

are not considered to be organ or tissue homing molecules within the invention.

TABLE 1

SUMMARY OF IN VIVO TARGETING OF VARIOUS ORGANS

ORGAN/MOTIF (SEQ ID NO:)	% OF MOTIF AMONG ALL CLONES	LUNG/BRAIN RATIO
GUT		
YSGKWGK (9)	22	30
GISALVLS (19)	11	nd
SRRQPLS (153)	11	2
MSPQLAT (159)	11	nd
MRRDEQR (172)		
QVRRVPE (155)		
VRRGSPQ (164)		
GGRGSWE (167)		
FRVRGSP (169)		
RVRGPER (165)		
LIVER		
VKSVCRT (12)	11	nd
WRQNMPL (418)	6	nd
SRRFVGG (406)	6	nd
ALERRSL (408)		
ARRGWTL (405)		
PROSTATE		
SMSIARL (21)	6	34
VSFLEYR (22)	6	17
RGRWLAL (279)	6	nd
ADRENAL GLAND		
LMLPRAD (27)	11	50
LPRYLLS (28)		
R(Y/F)LLAGG (404)		
RYPLAGG (389)		

ORGAN/MOTIF (SEQ ID NO:)	% OF MOTIF AMONG ALL CLONES	LUNG/BRAIN RATIO
OVARY		
EVRSRLS (10)	22	3
FFAAVRS (295)		
VRARLMS (301)		
RVGLVAR (11)	22	5
RVRLVNL (294)		
PANCREAS		
SWCEPGWCR (4)		20
SKIN	9	
CVALCREACGEGC (3)	6	7
CSSGCSKNCLEMC (181)		2
LUNG		
CTLRDRNC (15)	10	8
CGKRYRNC (20)	5	5
CLRPYLNC (45)	10	6
CGFELETC (2)	5	9
CIGEVEVC (16)	5	6
CKWSRLHSC (65)	11	3
CWRGDRKIC (56)	8	2
CERVVGSSC (59)	9	4
CLAKENVVC (13)	13	2
CTVNEAYKTRMC (75)	22	3
CRLRSYGTLSLC (76)	5	0.4
CRPWHNQAHTEC (82)	14	5
CGFECVRQCPERC (1)	40	60

The organ or tissue homing peptide molecules exemplified herein range in size from about 7 to 13 amino acids in length. However, based, for example, on the ability of the RGD integrin binding motif to mediate integrin binding by itself or when present in a large protein, it will be recognized that the organ homing molecules of the invention also can be expected to maintain their homing capability in the context of a significantly longer polypeptide sequence. Thus, an organ homing peptide of the invention can be at least three amino acids, generally at least six amino acids or seven amino acids or more, and can be significantly larger, for example, about 20 to 50 amino acids or 100 amino acids or more.

The invention provides lung homing peptides such as CGFECVRQCPERC (SEQ ID NO: 1) and CGFELETC (SEQ ID NO: 2), which share a GFE motif; CTLRDRNC (SEQ ID NO: 15); and CIGEVEVC (SEQ ID NO: 16; see Table 1), which contains an EVE motif that is similar to the ELE motif present in CGFELETC (SEQ ID NO: 2). The exemplified lung homing peptides were identified by injection of a $CX_3CX_3CX_3C$, (SEQ ID NO: 25), CX_7C (SEQ ID NO: 24) or CX_6C (SEQ ID NO: 26) cyclic library into mice (Example II). The lung homing peptides CGFECVRQCPERC (SEQ ID NO: 1) and CGFELETC (SEQ ID NO: 2) exhibited a 60-fold and 9-fold enrichment, respectively, as compared to unselected phage, with few phage detected in kidney or brain (Example II; see, also, Figures 1 and 2 and Table 1). In addition, the lung homing peptides CTLRDRNC (SEQ ID NO: 15) and CIGEVEVC (SEQ ID NO: 16) exhibited a 8-fold and 6-fold enrichment, respectively, over unselected phage (Table 1). Coinjection of a glutathione-S-transferase-(GST-)CGFECVRQCPERC (SEQ ID NO: 1) fusion peptide with

phage expressing the cognate CGFECVRQCPERC (SEQ ID NO: 1) peptide inhibited homing by 70%, and coinjection of GST-CGFELETC (SEQ ID NO: 2) with phage expressing (SEQ ID NO: 2) inhibited lung homing by 30% (Figure 3). Immunohistochemical staining of lung following administration of phage displaying a lung homing peptide to mice revealed staining within the alveolar capillaries. No apparent preference for homing of the phage to any particular region of the lung was observed; however, no staining was observed in bronchiolar walls or some larger blood vessels (Example III), or in many other tissues analyzed. These results indicate that *in vivo* panning can be used to identify and analyze endothelial cell specificities within lung, thus providing a means to differentially target lung.

The invention also provides skin homing peptides such as CVALCREACGEGC (SEQ ID NO: 3; Table 5), which were identified by injection of a $CX_3CX_3CX_3C$ (SEQ ID NO: 25) cyclic library into mice (Example II). The skin homing peptide sequence CVALCREACGEGC (SEQ ID NO: 3) exhibited a 7-fold selectivity for skin over unselected phage and over background in brain and kidney (Figure 2; see, also, Table 1). Coinjection of GST-CVALCREACGEGC (SEQ ID NO: 3) with phage expressing CVALCREACGEGC (SEQ ID NO: 3) inhibited homing to skin by 55%, whereas coinjection with GST, alone, had no effect on homing (see Figure 3B). Immunohistochemical staining of skin following administration of phage displaying a skin homing peptide revealed that staining was localized to the hypodermis; no staining was observed in the dermis (Example III).

The invention further provides pancreas homing peptides such as SWCEPGWCR (SEQ ID NO: 4; Table 3). The exemplified pancreas homing molecules were identified by injection of a CX_7C (SEQ ID NO: 24) or X_2CX_4CX (SEQ ID NO: 23) cyclic library into mice (Example II). The pancreas homing peptide SWCEPGWCR (SEQ ID NO: 4) exhibited a 20-fold selectivity for pancreas over unselected phage and over brain (Table 1; Figure 2). However, coinjection of GST-SWCEPGWCR (SEQ ID NO: 4) did not inhibit SWCEPGWCR (SEQ ID NO: 4) pancreas homing, presumably due to a conformational effect of GST on the pancreas homing peptide. Immunohistochemical staining of pancreas following administration of phage displaying a pancreas homing peptide revealed that staining was localized to the capillaries as well as larger blood vessels of the exocrine pancreas; no significant staining was observed in the endocrine vasculature (Example III). This result demonstrates that histologically and physiologically distinguishable regions within a particular organ can express unique target molecules, which provide a target for an organ homing molecule of the invention. Accordingly, the organ homing molecules of the invention provide a means to differentially targeted specific regions of a selected organ or tissue.

Retina homing peptides such as CSCFRDVCC (SEQ ID NO: 5) and CRDVVSVIC (SEQ ID NO: 6) also are provided (see Table 6). The exemplified retina homing molecules were identified by injection of a CX_7C (SEQ ID NO: 24) cyclic library into rats (Example II). The retina homing peptides CSCFRDVCC (SEQ ID NO: 5) and CRDVVSVIC (SEQ ID NO: 6), when injected individually with a control fdAMPLAY88 phage, exhibited a 3-fold and

2-fold enrichment, respectively, in retina (Example II). However, immunohistochemical staining revealed an absence of retina staining, presumably due to a relatively modest accumulation of the retina homing phage in the target tissue.

The invention also provides prostate homing peptides such as SMSIARL (SEQ ID NO: 21) and VSFLEYR (SEQ ID NO: 22), which were identified by injection of an X_7 (SEQ ID NO: 29) library into mice (Table 7). The peptides were isolated by the regular method. The prostate homing peptides SMSIARL (SEQ ID NO: 21) and VSFLEYR (SEQ ID NO: 22) exhibited a 34-fold and 17-fold enrichment, respectively, in prostate as compared to brain (Table 1).

Also provided are ovary homing peptides such as RVGLVAR (SEQ ID NO: 11) and EVRSRLS (SEQ ID NO: 10), which were identified by injection of an X_7 (SEQ ID NO: 29) library into mice (Table 8). The peptides were isolated by the regular method. The ovary homing peptides RVGLVAR (SEQ ID NO: 11) and EVRSRLS (SEQ ID NO: 10) each comprised 22% of 40 clones sequenced and exhibited a 5-fold and a 3-fold enrichment, respectively, in ovary as compared to brain (Table 1).

The invention also provides adrenal gland homing peptides such as LMLPRAD (SEQ ID NO: 27) and LPRYLLS (SEQ ID NO: 28), which share a LPR motif (see Table 11), or the peptides R(Y/F)LLAGG (SEQ ID NO: 404) and RYPLAGG (SEQ ID NO: 389), which share the motif LAGG (SEQ ID NO: 430; see Table 10). The exemplified adrenal gland homing peptides were identified by injection of an X_7 (SEQ ID NO: 29) library into mice. The peptides were isolated by the regular method. The

adrenal gland homing peptide LMLPRAD (SEQ ID NO: 27) exhibited a 50-fold enrichment in adrenal gland as compared to brain (Table 1).

Also provided are liver homing peptides. Such peptides were identified by injection of an X_7 (SEQ ID NO: 29) library into mice. The peptides were isolated by the regular method (see Example II, Table 1, and Table 11, below).

In addition, lymph node homing peptides, such as AGCSVTVCG (SEQ ID NO: 315) are provided (Table 9, below). Such peptides were identified by injection of an X_2CX_4CX (SEQ ID NO: 23) library into mice. The peptides were isolated by the regular method.

The invention also provides gut homing peptides such as YSGKWGK (SEQ ID NO: 9) and YSGKWGW (SEQ ID NO: 156), which were identified by injection of an X_7 (SEQ ID NO: 29) library into mice (Tables 1 and 4) and differ only in the last amino acid position. The peptides were isolated by the regular method. The gut homing peptide YSGKWGK (SEQ ID NO: 9) was present in 22% of 40 clones sequenced and was enriched 30-fold in gut as compared to brain (Table 1). In addition, gut homing peptides such as QVRRVPE (SEQ ID NO: 155) and VRRGSPQ (SEQ ID NO: 164), which share a VRR motif, were identified, as were the peptides VRRGSPQ (SEQ ID NO: 164), GGRGSWE (SEQ ID NO: 167) and FRVRGSP (SEQ ID NO: 169), which share an RGS motif.

The organ homing molecules of the invention are particularly useful as conjugates, which comprise the organ homing molecule linked to a moiety. Thus, a lung, skin, pancreas, retina, prostate, ovary, lymph

node, adrenal gland, liver or gut homing molecule of the invention can be linked to a moiety, such conjugates being useful for directing the moiety to the particular selected organ.

As used herein, the term "moiety" is used broadly to mean a physical, chemical, or biological material that is linked to an organ or tissue homing molecule. Generally, a moiety linked to an organ homing molecule imparts a biologically useful function to the homing molecule. A moiety can consist of any natural or nonnatural material for example, peptide or polypeptide sequences, organic or inorganic molecules or compositions, nucleic acid molecules, carbohydrates, lipids or combinations thereof.

A moiety can be a physical, chemical or biological material such as a virus, viral gene therapy vector, cell, liposome, microcapsule, micropump or other chambered microdevice, which can be used, for example, as a drug delivery system. Generally, such microdevices should be biologically inert and, if desired, biodegradable or excretable. Various moieties, including microcapsules, which can contain an agent, and methods for linking a moiety or chambered microdevice to an organic molecule of the invention are well known in the art and commercially available (see, for example, "Remington's Pharmaceutical Sciences" 18th ed. (Mack Publishing Co. 1990), chapters 89-91; Harlow and Lane, Antibodies: A laboratory manual (Cold Spring Harbor Laboratory Press 1988), each of which is incorporated herein by reference; see, also, Hermanson, *supra*, 1996). Additional examples of moieties are known to those skilled in the art and are intended to be included within the meaning of the term so long as

they possess a biologically useful function when linked to the homing molecules of the invention.

Linking of a moiety to an organ homing molecule for the purpose of directing the moiety to the selected organ or tissue was demonstrated by the linking of a brain homing peptide to a red blood cell (RBC), wherein the peptide directed homing of the RBC to the brain (U.S. Patent No. 5,622,699, *supra*, 1997). These results indicate that an organ or tissue homing molecule of the invention can be linked to another moiety in order to direct the moiety to a selected organ or tissue. For example, a liver homing molecule or a lung homing molecule can be linked to a nucleic acid encoding the CFTR gene and upon administration to a subject, expression of CFTR is targeted to the liver or to the lung, respectively. Similarly, a lung homing molecule can be linked to a protease inhibitor such that, upon administration of the conjugate comprising the lung homing molecule and the protease inhibitor to a subject, the protease inhibitor is targeted to the lung. Such a conjugate can be useful, for example, for treating a subject suffering from emphysema, which is characterized by excessive protease production in the lungs and autodigestion of the organ.

An organ and tissue homing molecule of the invention can be useful for directing to a selected organ or tissue a therapeutic agent, diagnostic agent or imaging agent, a tag or insoluble support, a liposome or a microcapsule comprising, for example, a permeable or semipermeable membrane, wherein an agent such as a drug to be delivered to a selected organ or tissue is contained within the liposome or microcapsule. These and other moieties known in the

art can be used in a conjugate of the invention, and in a method of the invention, as disclosed herein.

In one embodiment, a moiety can be a detectable agent such as a radionuclide or an imaging agent, which allows detection or visualization of the selected organ or tissue. Thus, the invention provides a conjugate comprising a lung, skin, pancreas, retina, prostate, ovary, lymph node, adrenal gland, liver or gut homing molecule, linked to a detectable agent. The type of detectable agent selected will depend upon the application. For example, for an *in vivo* diagnostic imaging study of the lung in a subject, a lung homing molecule can be linked to an agent that, upon administration to the subject, is detectable external to the subject. For detection of such internal organs or tissues, for example, the prostate, a gamma ray emitting radionuclide such as indium-113, indium-115 or technetium-99 can be linked to a prostate homing molecule and, following administration to a subject, can be visualized using a solid scintillation detector. Alternatively, for organs or tissues at or near the external surface of a subject, for example, retina, a fluorescein-labeled retina homing molecule can be used such that the endothelial structure of the retina can be visualized using an opthalamoscope and the appropriate optical system.

Molecules that selectively home to a pathological lesion in an organ or tissue similarly can be linked to an appropriate detectable agent such that the size and distribution of the lesion can be visualized. For example, where an organ or tissue homing molecule homes to a normal organ or tissue, but not to a pathological lesion in the organ or tissue,

the presence of the pathological lesion can be detected by identifying an abnormal or atypical image of the organ or tissue, for example, the absence of the detectable agent in the region of the lesion.

A detectable agent also can be an agent that facilitates detection *in vitro*. For example, a conjugate comprising a homing molecule linked to an enzyme, which produces a visible signal when an appropriate substrate is present, can detect the presence of an organ or tissue to which the homing molecule is directed. Such a conjugate, which can comprise, for example, alkaline phosphatase or luciferase or the like, can be useful in a method such as immunohistochemistry. Such a conjugate also can be used to detect the presence of a target molecule, to which the organ homing molecule binds, in a sample, for example, during purification of the target molecule.

In another embodiment, a moiety can be a therapeutic agent. Thus, the invention provides a conjugate comprising a lung, skin, pancreas, retina, prostate, ovary, lymph node, adrenal gland, liver or gut homing molecule linked to a therapeutic agent.

A therapeutic agent can be any biologically useful agent that, when linked to an organ homing molecule of the invention, exerts its function at the site of the selected organ or tissue. For example, a therapeutic agent can be a small organic molecule that, upon binding to a target cell due to the linked organ homing molecule, is internalized by the cell where it can effect its function. A therapeutic agent can be a nucleic acid molecule that encodes a protein involved in stimulating or inhibiting cell survival, cell

proliferation or cell death, as desired, in the selected organ or tissue. For example, a nucleic acid molecule encoding a protein such as Bcl-2, which inhibits apoptosis, can be used to promote cell survival, whereas a nucleic acid molecule encoding a protein such as Bax, which stimulates apoptosis, can be used to promote cell death of a target cell.

A particularly useful therapeutic agent that stimulates cell death is ricin, which, when linked to an organ homing molecule of the invention, can be useful for treating a hyperproliferative disorder, for example, cancer. A conjugate comprising an organ homing molecule of the invention and an antibiotic, such as ampicillin or an antiviral agent such as ribavirin, for example, can be useful for treating a bacterial or viral infection in a selected organ or tissue.

A therapeutic agent also can inhibit or promote the production or activity of a biological molecule, the expression or deficiency of which is associated with the pathology. Thus, a protease inhibitor can be a therapeutic agent that, when linked to an organ homing molecule, can inhibit protease activity at the selected organ or tissue, for example, the pancreas. A gene or functional equivalent thereof such as a cDNA, which can replenish or restore production of a protein in a selected organ or tissue, also can be a therapeutic agent useful for ameliorating the severity of a pathology. A therapeutic agent also can be an antisense nucleic acid molecule, the expression of which inhibits production of a deleterious protein, or can be a nucleic acid molecule encoding a dominant negative protein or a fragment

thereof, which can inhibit the activity of a deleterious protein.

In another embodiment, the invention provides a conjugate comprising a lung, skin, pancreas, retina, prostate, ovary, lymph node, adrenal gland, liver or gut homing molecule linked to a tag. A tag can be, for example, an insoluble support such as a chromatography matrix, or a molecule such as biotin, hemagglutinin antigen, polyhistidine, T7 or other molecules known in the art. Such a conjugate comprising a tag can be useful to isolate a target molecule, to which the organ homing molecule binds.

When administered to a subject, a conjugate comprising an organ homing molecule and a moiety is administered as a pharmaceutical composition containing, for example, the conjugate and a pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers are well known in the art and include, for example, aqueous solutions such as water or physiologically buffered saline or other solvents or vehicles such as glycols, glycerol, oils such as olive oil or injectable organic esters.

A pharmaceutically acceptable carrier can contain physiologically acceptable compounds that act, for example, to stabilize or to increase the absorption of the complex. Such physiologically acceptable compounds include, for example, carbohydrates, such as glucose, sucrose or dextrans, antioxidants, such as ascorbic acid or glutathione, chelating agents, low molecular weight proteins or other stabilizers or excipients. One skilled in the art would know that the choice of a pharmaceutically acceptable carrier,

including a physiologically acceptable compound, depends, for example, on the route of administration of the composition. The pharmaceutical composition also can contain an agent such as a cancer therapeutic agent or other therapeutic agent as desired.

One skilled in the art would know that a pharmaceutical composition containing an organ homing molecule can be administered to a subject by various routes including, for example, orally or parenterally, such as intravenously. The composition can be administered by injection or by intubation. The pharmaceutical composition also can be an organ homing molecule linked to a moiety such as a liposome or other polymer matrix, which can have incorporated therein, for example, a drug that promotes or inhibits cell death (Gregoriadis, Liposome Technology, Vol. 1 (CRC Press, Boca Raton, FL 1984), which is incorporated herein by reference). Liposomes, for example, which consist of phospholipids or other lipids, are nontoxic, physiologically acceptable and metabolizable carriers that are relatively simple to make and administer.

In performing a diagnostic or therapeutic method as disclosed herein, an effective amount of a conjugate comprising an organ homing molecule must be administered to the subject. An "effective amount" is the amount of the conjugate that produces a desired effect. An effective amount will depend, for example, on the moiety linked to the organ homing molecule and on the intended use. For example, a lesser amount of a radiolabeled homing molecule can be required for imaging as compared to the amount of the radiolabeled molecule administered for therapeutic purposes, where

cell killing is desired. An effective amount of a particular conjugate for a specific purpose can be determined using methods well known to those in the art.

The route of administration of an organ molecule will depend, in part, on the chemical structure of the organ homing molecule. Peptides, for example, are not particularly useful when administered orally because they can be degraded in the digestive tract. However, methods for chemically modifying peptides to render them less susceptible to degradation by endogenous proteases or more absorbable through the alimentary tract are well known (see, for example, Blondelle et al., *supra*, 1995; Ecker and Crooke, *supra*, 1995; Goodman and Ro, *supra*, 1995). Such methods can be performed on peptides that home to a selected organ or tissue. In addition, methods for preparing libraries of peptide analogs such as peptides containing D-amino acids; peptidomimetics consisting of organic molecules that mimic the structure of a peptide; or peptoids such as vinylogous peptoids, have been previously described above and can be used to identify homing molecules suitable for oral administration to a subject.

The invention provides methods of identifying a selected organ or tissue by administering to a subject a conjugate comprising a lung, skin, pancreas, retina, prostate, ovary, lymph node, adrenal gland, liver or gut and a detectable agent. A conjugate comprising an organ homing molecule of the invention linked to a detectable moiety conjugate can be administered to a subject and used to identify or visualize a selected organ or tissue. The ability to

visualize an organ, particularly an internal organ, provides a means diagnose a pathology of the selected organ or tissue. For example, a prostate homing molecule linked to indium-113 can be administered to a subject in order to image the prostate. Such a method can be particularly valuable because methods for imaging the prostate are limited. The presence of a prostate pathology can be revealed by detecting that a region of the prostate does not contain the conjugate, thus indicating an abnormality in circulation to the region, or by detecting that the prostate is abnormally enlarged or lacking its normal boundaries. For organs or tissues such as retina, which can be visualized directly using an ophthalmoscope, a conjugate comprising a retina homing molecule linked to fluorescein can be administered to a subject and used to examine the vascular integrity and circulation in the retina. The absence of a normal or typical pattern of retinal image can indicate the presence of a retinal pathology in the region. For example, an abnormal retinal pattern can reflect vascular changes indicative of a hyperproliferative or degenerative pathology.

In principle, an organ homing molecule of the invention can have an inherent biological property, such that administration of the molecule provides direct biological effect. For example, an organ homing molecule can be sufficiently similar to a naturally occurring ligand for the target molecule that the organ homing molecule mimics the activity of the natural ligand. Such an organ homing molecule can be useful as a therapeutic agent having the activity of the natural ligand. For example, where the organ homing molecule mimics the activity of a growth factor that binds a receptor expressed by the selected organ or tissue,

such as a skin homing molecule that mimics the activity of epidermal growth factor, administration of the organ homing molecule can result in cell proliferation in the organ or tissue. Such inherent biological activity of an organ homing molecule of the invention can be identified by contacting the cells of the selected organ or tissue with the homing molecule and examining the cells for evidence of a biological effect, for example, cell proliferation or, where the inherent activity is a toxic effect, cell death.

In addition, an organ homing molecule of the invention can have an inherent activity of binding a particular target molecule such that a corresponding ligand cannot bind the receptor. It is known, for example, that various types of cancer cells metastasize to specific organs or tissues, indicating that the cancer cells express a ligand that binds a target molecule in the organ to which it metastasizes. Thus, administration of a lung homing molecule, for example, to a subject having a tumor that metastasizes to lung, can provide a means to prevent the potentially metastatic cancer cell from becoming established in the lung. In general, however, the organ homing molecules of the invention are particularly useful for targeting a moiety to a selected organ or tissue, particularly to lung, skin, pancreas, retina, prostate, ovary, lymph node, adrenal gland, liver or gut. Thus, the invention provides methods of treating a pathology in a selected organ or tissue by administering to a subject having the pathology a conjugate comprising an organ homing molecule of the invention linked to a therapeutic agent.

Specific disorders of the lung, for example, can be treated by administering to a subject a conjugate comprising a lung homing molecule linked to a therapeutic agent. Since a lung homing molecule of the invention can localize to the capillaries and alveoli of the lung, disorders associated with these regions are especially amenable to treatment with a conjugate comprising the lung homing molecule. For example, bacterial pneumonia often originates in the alveoli and capillaries of the lung (Rubin and Farber, Pathology 2nd ed., (Lippincott Co.,1994)). Thus, a lung homing molecule conjugated to a suitable antibiotic can be administered to a subject to treat the pneumonia. Similarly, cystic fibrosis causes pathological lesions in the lung due to a defect in the CFTR. Thus, administration of a lung homing molecule conjugated to a nucleic acid molecule encoding the CFTR provides a means for directing the nucleic acid molecule to the lung as an *in vivo* gene therapy treatment method.

The invention also provides methods of treating a pathology of the skin by administering to a subject having the pathology a conjugate comprising a skin homing molecule and a therapeutic agent. For example, a burn victim can be administered a conjugate comprising a skin homing molecule linked to epithelial growth factor or platelet derived growth factor such that the growth factor is localized to the skin where it can accelerate regeneration or repair of the epithelium and underlying dermis. Furthermore, a method of the invention can be useful for treating skin pathologies caused by bacterial infections, particularly infections that spread through the hypodermis and dermis or that are localized in these regions, by administering to a subject a conjugate

comprising a skin homing molecule linked to an antibiotic.

The invention also provides methods of treating a pathology of the pancreas by administering to a subject having the pathology a conjugate comprising a pancreas homing molecule linked to a therapeutic agent. In particular, since a pancreas homing molecule of the invention can localize to the exocrine pancreas, a pathology associated with the exocrine pancreas can be treated and, in some cases, may not adversely affect the endocrine pancreas. A method of the invention can be particularly useful to treat acute pancreatitis, which is an inflammatory condition of the exocrine pancreas caused by secreted proteases damaging the organ. A conjugate comprising a pancreas homing molecule linked to a protease inhibitor can be used to inhibit the protease mediated destruction of the tissue, thus reducing the severity of the pathology. Appropriate protease inhibitors useful in such a conjugate are those that inhibit enzymes associated with pancreatitis, including, for example, inhibitors of trypsin, chymotrypsin, elastase, carboxypeptidase and pancreatic lipase. A method of the invention also can be used to treat a subject having a pancreatic cancer, for example, ductal adenocarcinoma, by administering to the subject a conjugate comprising a therapeutic agent linked to a molecule that homes to pancreas.

The methods of the invention also can be used to treat a pathology of the eye, particularly the retina, by administering to a subject having the pathology a conjugate comprising a retina homing molecule linked to a therapeutic agent. For example,

proliferative retinopathy is associated with neovascularization of the retina in response to retinal ischemia due, for example, to diabetes. Thus, administration of a conjugate comprising a retina homing molecule linked to a gene that stimulates apoptosis, for example, Bax, can be used to treat the proliferative retinopathy. Similarly, methods of the invention can be used to diagnose or treat prostate, ovary, lymph node, adrenal gland, liver, or gut pathology using the appropriate organ or tissue homing molecules disclosed herein either alone, or linked to a desired moiety.

An organ or tissue homing molecule is useful, for example, for targeting a therapeutic or detectable agent to the selected organ or tissue. In addition, an organ or tissue homing molecule can be used to identify the presence of a target molecule in a sample. As used herein, the term "sample" is used in its broadest sense to mean a cell, tissue, organ or portion thereof that is isolated from the body. A sample can be, for example, a histologic section or a specimen obtained by biopsy or cells that are placed in or adapted to tissue culture. If desired, a sample can be processed, for example, by homogenization, which can be an initial step for isolating the target molecule to which an organ or tissue homing molecule binds.

An organ homing molecule obtained as disclosed herein can be useful for identifying the presence of a target molecule, particularly a cell surface protein, that is recognized by the homing molecule, or for substantially isolating the target molecule. Thus, the invention provides methods of identifying target molecules that selectively bind a

lung homing molecule, a skin homing molecule, a pancreas homing molecule, a retina homing molecule, a prostate homing molecule, an ovary homing molecule, a lymph node homing molecule, an adrenal gland homing molecule, a liver homing molecule or a gut homing molecule. Such a method comprises contacting a sample of the selected organ or tissue, for example, prostate, with a prostate homing molecule, and detecting selective binding of a component of a sample, wherein such binding identifies the presence of a target molecule.

An organ or tissue homing molecule such as a prostate homing peptide can be linked to a tag, for example, a solid support such as a chromatography matrix. The immobilized organ homing molecule then can be used for affinity chromatography by passing an appropriately processed sample of prostate tissue over a column containing the matrix under conditions that allow specific binding of the prostate homing molecule to the particular target molecule (see, for example, Deutshcer, Meth. Enzymol., Guide to Protein Purification (Academic Press, Inc., ed. M.P. Deutscher, 1990), Vol. 182, which is incorporated herein by reference; see, for example, pages 357-379). Unbound and nonspecifically bound material can be removed and the target molecule, which forms a complex with the prostate homing molecule, can be eluted from the column and collected in a substantially isolated form. The substantially isolated prostate target molecule then can be characterized using well known methods. An organ or tissue homing molecule also can be linked to a detectable agent such as a radionuclide, a fluorescent molecule, an enzyme or a labeled biotin tag and can be used, for example, to screen a sample in order to

detect the presence of the target molecule or to follow the target molecule during its isolation.

As an alternative to using an organ or tissue sample to identify a target molecule of the selected organ or tissue, extracts of cultured cells derived from the selected organ or tissue, or extracts of cultured endothelial cells can be used as the starting material. Selection of cells containing the target molecule can be determined by using binding and cell attachment assays (see Barry et al., Nature Med. 2:299-305 1996), which is incorporated herein by reference). Those cells containing the target molecule can be used to prepare extracts for the isolation and identification of a target molecule, as described above.

Upon identifying an appropriate cell line expressing the target molecule, the target molecule can be labeled by growing the cells in medium containing radiolabeled amino acids. The radiolabeled amino acids are incorporated into the target molecule, thus facilitating its identification during purification. Labeled cells then can be extracted with octylglucoside and the extract can be fractionated by affinity chromatography using a pancreas homing molecule coupled to a matrix such as SEPHAROSE. Extracts prepared, for example, from human umbilical vein endothelial cells can be used as a control. The purified target molecule then can be microsequenced and antibodies can be prepared. If desired, oligonucleotide probes can be prepared and used to isolate cDNA clones encoding the target receptor. Alternatively, an anti-receptor antibody can be used to isolate a cDNA clone from an expression library (see Argraves et al., J. Cell Biol.

105:1183-1190 (1987), which is incorporated herein by reference).

In addition to biochemically isolating a target molecule, a nucleic acid encoding the target molecule can be isolated by using, for example, a pancreas homing molecule as a chemical probe to screen a pancreatic cDNA expression library for clones that express the target molecule. For example, bacteria expressing a pancreatic cDNA library can be attached to a membrane, lysed, and screened with a pancreas homing molecule conjugated, for example, to an enzyme that produces a colorimetric or fluorescent signal. Bacterial clones expressing a target molecule are identified and the cDNA encoding the target molecule can be isolated. Additionally, a mammalian cell expression cloning system such as the COS cell system can be used to identify a target molecule. For example, a cDNA library can be prepared using mRNA from primary pancreas cells which can be cloned into an expression vector. Cells expressing a cDNA encoding the target molecule then can be selected using the pancreas homing peptide as a probe, for example, by panning of cell clones against pancreas homing peptide attached to a plate. Alternatively, phage can be used to display the pancreas homing peptide and can be attached to magnetic beads coated, for example, with anti-M13 antibodies (Pharmacia). Cells expressing the target molecule that bind to the pancreas homing peptide then can be recovered and the plasmids encoding the receptor can be isolated. The recovered plasmid preparations can be divided into pools and examined in COS cell transfections. The procedure can be repeated until single plasmids are obtained that enable the COS cells to bind the pancreas homing peptide.

The following examples are intended to illustrate but not limit the present invention.

EXAMPLE I

IN VIVO PANNING

This example demonstrates methods for preparing a phage display library and screening the library using *in vivo* panning to identify phage expressing peptides that home to a selected organ or tissue.

A. Preparation of phage libraries:

Phage display libraries were constructed using the fuse 5 vector as described by Koivunen et al., *supra*, 1995; see, also, Koivunen et al., *supra*, 1994b). Libraries encoding peptides designated CX_6C (SEQ ID NO: 26), CX_7C (SEQ ID NO: 24), $CX_{10}C$ (SEQ ID NO: 30)) $CX_3CX_3CX_3C$ (SEQ ID NO: 25), X_2CX_4CX (SEQ ID NO: 23), and X_7 (SEQ ID NO: 29), were prepared, where "C" indicates cysteine and "X_N" indicates the given number of individually selected amino acids. These libraries can display cyclic peptides when at least two cysteine residues are present in the peptide.

The libraries containing the defined cysteine residues were generated using oligonucleotides constructed such that "C" was encoded by the codon TGT and "X_N" was encoded by NNK, where "N" is equal molar mixtures of A, C, G and T, and where "K" is equal molar mixtures of G and T. Thus, the peptide represented by CX_6C (SEQ ID NO: 26) can be represented by an oligonucleotide having the sequence $TGT(NNK)_6TGT$ (SEQ ID NO: 31). Oligonucleotides were made double stranded

by 3 cycles of PCR amplification, purified and ligated to the nucleic acid encoding the gene III protein in the fuse 5 vector such that, upon expression, the peptide is present as a fusion protein at the N-terminus of the gene III protein.

The vectors were transfected by electroporation into MC1061 cells. Bacteria were cultured for 24 hr in the presence of 20 µg/ml tetracycline, then phage were collected from the supernatant by precipitation twice using polyethylene glycol. Each library contained about 10^{12} transducing units/ml (TU; individual recombinant phage).

B. *In vivo* panning of phage:

For lung and pancreas, a mixture of phage libraries containing 10^{10} TU was diluted in 200 µl DMEM and injected into the tail vein of anesthetized BALB/c mice (2 month old; Harlan Sprague Dawley; San Diego CA); AVERTIN (0.017 ml/g) was used as anesthetic (Pasqualini and Ruoslahti, *supra*, 1996). After 1-4 minutes, mice were snap frozen in liquid nitrogen or, after about 5 minutes of phage circulation, the mice were perfused through the heart with 5-10 ml of DMEM (Sigma; St. Louis MO). To recover the phage, the organs from the perfused mice or partially thawed organs from snap frozen mice were collected and weighed, then were homogenized in 1 ml DMEM-PI (DMEM containing protease inhibitors (PI); phenylmethyl sulfonyl fluoride (PMSF; 1 mM), aprotinin (20 µg/ml), leupeptin (1 µg/ml)).

Organ samples were washed 3 times with ice cold DMEM-PI containing 1% bovine serum albumin (BSA),

then directly incubated with 1 ml K91-kan bacteria for 1 hr. Ten ml NZY medium containing 0.2 µg/ml tetracycline (NZY/tet) was added to the bacterial culture, the mixture was incubated in a 37°C shaker for 1 hr, then 200 µl aliquots were plated in agar plates containing 40 µg/ml tetracycline (tet/agar).

For *in vivo* panning of skin, two month old BALB/c nude mice were used to avoid contamination by hair. The mice were injected intravenously with phage as described above and, after perfusion through the heart, the skin was removed in large sections and placed on an ice cold plate with the hypodermis facing up. The skin was scraped with a scalpel to remove mostly hypodermis, which was then processed for phage recovery as described below.

For *in vivo* panning of retina, two month old female Simonson Albino rats were used to provide larger tissue samples than mice. The rats were anesthetized with phenobarbital (50 mg/kg body weight), and, while under deep anesthesia, the abdominal cavity of the rats was opened and 10^{10} TU of a phage library was injected into the left ventricle of the heart through the diaphragm. After 2-5 minutes of phage circulation, the eyes were removed, then washed once in 70% EtOH and once in PBS. The anterior chamber, with cornea and lens, was removed and the retina was peeled from the remaining posterior chamber. The tissue was weighed, homogenized with a syringe bulb in 1 ml of ice cold DMEM containing protease inhibitors (1 mM PMSF, 20 µg/ml aprotinin and 1 µg/ml of leupeptin; all from Sigma; St. Louis MO). The tissue was washed 3 times with 1 ml of DMEM and the phage were rescued as described below.

Approximately 250 to 300 individual bacterial colonies containing phage recovered from the various organs or tissues were grown for 16 hr in 5 ml NZY/tet. In some experiments, approximately 1000 individual bacteria containing phage were picked and the phage were amplified in 2 ml of NZY/tet or the entire plate containing phage was scraped, pooled and grown in bulk and processed for injection. Where phage were cultured separately, the cultures were pooled and the phage were injected into mice or rats as described above for a second round of *in vivo* panning. In some experiments, a third or fourth round of panning was performed. Phage DNA was purified from individual bacterial colonies obtained and the DNA sequences encoding the peptides expressed by selected phage were determined (see Koivunen et al., *supra*, 1994b).

EXAMPLE II

CHARACTERIZATION OF PEPTIDES THAT HOME TO A SELECTED ORGAN

This example demonstrates that an organ or tissue homing peptide of the invention selectively homes to a selected organ or tissue including an organ containing a component of the RES.

A. Lung is the selected organ

After two or three rounds of *in vivo* panning of mice injected with a cyclic $CX_3CX_3CX_3C$ (SEQ ID NO: 25) or a cyclic CX_6C (SEQ ID NO: 26) phage display library, four peptides that homed to lung were identified. The peptide sequences CGFECVRQCPERC (SEQ ID NO: 1; GFE-1) and CGFELETC (SEQ ID NO: 2; GFE-2) appeared repeatedly in the lung and two peptide

sequences from the CX_6C (SEQ ID NO: 26) library CTLRDRNC (SEQ ID NO: 15) and CIGEVEVC (SEQ ID NO: 16) also were found to home to lung (see Table 2, below).

To determine the specificity of lung homing of the individual peptides identified, phage displaying the peptides were amplified individually, diluted to the same input titer and administered to mice. Following administration, control kidney and brain organ was removed and the number of TU of phage in lung, kidney and brain was determined. The results shown in Figure 2 reveal that 10x and 35x more phage having the peptide sequence CGFECVRQCPERC (SEQ ID NO: 1; GFE-1) bound to lung than to kidney and brain, respectively. Figure 2 also reveals that CGFELETC (SEQ ID NO: 2; GFE-2) was found in lung at a 12x and 20x greater level than in kidney and brain, respectively. The lung homing peptides CGFECVRQCPERC (SEQ ID NO: 1; GFE-1), CGFELETC (SEQ ID NO: 2; GFE-2), CTLRDRNC (SEQ ID NO: 15) and CIGEVEVC (SEQ ID NO: 16) are enriched in lung at 35x, 9x, 6x and 5x, respectively, over unselected phage (see Figure 2). Thus, substantial enrichment of phage binding to the lung was observed in comparison to control brain and kidney and in comparison to unselected phage.

Specificity for the lung homing peptides was also determined by competition experiments with GST-fusion peptides. A GST-GFE-1 (SEQ ID NO: 1) fusion peptide coadministered with GFE-1 (SEQ ID NO: 1) inhibited GFE-1 (SEQ ID NO: 1) homing to the lung, whereas GST had no effect on homing (Figure 3A). In addition, the inhibitory effect of the GST-GFE-1 (SEQ ID NO: 1) on homing was dose dependent; 70% inhibition of homing occurred when injecting 500 µg of the

GST-GFE-1 (SEQ ID NO: 1) fusion protein (Figure 3B). Coinjection of GST-GFE-2 (SEQ ID NO: 2) with GFE-2 (SEQ ID NO: 2) inhibited homing to a lesser extent; 30% inhibition of homing occurred when injecting 500 µg of the GST-GFE-2 (SEQ ID NO: 2) fusion protein (Figure 3B). Interestingly, the GST-GFE-1 (SEQ ID NO: 1) fusion was more efficient at inhibiting GFE-2 (SEQ ID NO: 2) homing to the lung; 60% inhibition of GFE-2 (SEQ ID NO: 2) homing occurred when injecting 500 µg of the GST-GFE-1 (SEQ ID NO: 1) fusion protein (Figure 3B). However, no inhibitory effect of GFE-1 (SEQ ID NO: 1) homing was observed when coinjecting GST-GFE-2 (SEQ ID NO: 2). This can be explained by GFE-1 (SEQ ID NO: 1) having a higher affinity for a shared target molecule than GFE-2 (SEQ ID NO: 2).

Additional lung homing peptides were obtained and the amino acid sequences were determined for the inserts (see Table 2). Peptides containing a GFE motif predominated (see Table 1; SEQ ID NOS: 1 and 2). Other peptides that were present more than once in lung are indicated by an asterisk in Table 2 (below), and the remaining peptides were identified one time each.

These results indicate that the selection of the peptides containing the GFE motif represents the selective binding of several independent phage displaying peptides having the GFE sequence and is not an artifact due, for example, to phage amplification. In addition, in some cases, phage that expressed peptides having the same amino acid sequence were encoded by oligonucleotides having different sequences, therefore confirming that homing of a particular phage to a lung is due to the specific peptide expressed on the phage.

These results demonstrate that *in vivo* panning can be used to screen phage display libraries in order to identify phage expressing peptides that home to lung, which contain a component of the RES.

B. Skin is the selected tissue:

After two or three rounds of *in vivo* panning of mice injected with a cyclic $CX_3CX_3CX_3C$ (SEQ ID NO: 25) phage display library, the peptide sequence CVALCREACGEGC (SEQ ID NO: 3), which appeared repeatedly in skin, was identified (Table 1). To determine the specificity of skin homing of the sequence CVALCREACGEGC (SEQ ID NO: 3), phage displaying the peptide was amplified individually, diluted to the same input titer and administered to mice. Following administration, control kidney and brain organ were removed and the number of TU of phage in skin, kidney and brain was determined.

The results revealed that 7x more phage displaying the peptide sequence CVALCREACGEGC (SEQ ID NO: 3) bound to skin than to kidney or brain (see Figure 2; Table 1). The peptide CVALCREACGEGC (SEQ ID NO: 3) was enriched in skin 7x over unselected phage (Figure 2). Thus, substantial enrichment of phage binding to the skin was observed in comparison to control brain and kidney and in comparison to unselected phage. Additional skin homing peptides were obtained and the amino acid sequences were determined for the inserts (Table 5, below). Peptides that were identified more than one time during screening are indicated by an asterisk.

Specificity for the skin homing peptides was also determined by competition experiments with GST-fusion peptides. Figure 3B shows that a GST-CVALCREACGEGC (SEQ ID NO: 3) fusion peptide coadministered with CVALCREACGEGC (SEQ ID NO: 3) inhibited homing to the skin, whereas GST had no effect on homing. The inhibitory effect of the GST-GFE-1 on homing was about 55% when injecting 500 µg of the GST-CVALCREACGEGC (SEQ ID NO: 3) fusion protein (Figure 3B).

These results demonstrate that *in vivo* panning can be used to screen phage display libraries in order to identify phage expressing peptides that home to skin and that such homing is specific.

C. Pancreas is the selected organ:

After two or three rounds of *in vivo* panning of mice injected with a cyclic CX_7C (SEQ ID NO: 24) phage display library, various pancreas homing peptides were identified (Table 3). In particular, the peptide sequence SWCEPGWCR (SEQ ID NO: 4) appeared repeatedly in the pancreas. To determine the specificity of SWCEPGWCR (SEQ ID NO: 4), a phage displaying the sequence was amplified individually, diluted to the same input titer and administered to mice. Following administration, control brain organ was removed and the number of TU of phage in each pancreas and was determined. The results shown in Figure 2, reveal that 10x more phage displaying the peptide sequence SWCEPGWCR (SEQ ID NO: 4) bound to pancreas than to brain and additional experiments revealed up to 20x enrichment in pancreas as compared to brain (Table 1). In addition, SWCEPGWCR (SEQ ID NO: 4) exhibited a 22x

enrichment of phage to the pancreas as compared to unselected phage (see Figure 2). Thus, substantial enrichment of phage binding to the pancreas was observed in comparison to control tissue (brain) and to unselected phage.

These results demonstrate that *in vivo* panning can be used to identify molecules that selectively home to pancreas. In addition, the results indicate that *in vivo* panning identifies independent phage encoding the same peptide.

D. Retina is the selected tissue

Rats injected with a cyclic CX_7C (SEQ ID NO: 24) phage display library were subjected to *in vivo* panning and, after three rounds, the peptide sequences CSCFRDVCC (SEQ ID NO: 5) and CRDVVSVIC (SEQ ID NO: 6) were identified in retina. Because of small tissue sample size, the phage isolated could not be accurately quantitated. Thus, the selectivity of phage displaying the peptides was determined by individually amplifying the phage displaying the sequence and administering the phage to rats with a control phage fdAMPLAY88. This fd-ampicillin phage is similar to fd-tetracycline (fuse 5-based) in that it has the same infectivity.

Rats were injected with an equal amount of the CSCFRDVCC (SEQ ID NO: 5) or CRDVVSVIC (SEQ ID NO: 6) and the fdAMPLAY88 phage. Following administration, homing to retina was evaluated by comparing the number of TU of the selected phage on tetracycline plates and fdAMPLAY88 on ampicillin plates recovered from retina.

The results revealed that CSCFRDVCC (SEQ ID NO: 5) showed a 3x enrichment and CRDVVSVIC (SEQ ID NO: 6) showed a 2x enrichment in retina compared to control fdAMPLAY88 phage. Thus, substantial enrichment of phage binding to the retina was observed in comparison to control phage.

Additional retina homing peptides were obtained and the amino acid sequences were determined for the inserts (Table 6, below). Peptides that appeared more than one time are indicated. In particular, the RDV tripeptide motif was present in several different sequence contexts, indicating that the nucleic acids encoding the peptides were derived from a number of independent phage.

These results indicate that the selection of the peptides containing the RDV motif represents the selective binding of several independent phage displaying peptides having the RDV sequence and is not an artifact due, for example, to phage amplification. In addition, in some cases, phage that expressed peptides having the same amino acid sequence were encoded by oligonucleotides having different sequences, therefore confirming that homing of a particular phage to retina is due to the specific peptide expressed on the phage.

These results further demonstrate that the *in vivo* panning method is a generally applicable method for screening a library to identify, for example, phage expressing peptides that home to a selected organ or tissue, including organs and tissues containing a component of the RES. Database searches did not reveal any significant homology of the pancreas, lung, skin or

retina homing peptides to known ligands for endothelial cell receptors.

EXAMPLE III

IMMUNOHISTOLOGIC ANALYSIS OF LUNG, PANCREAS AND SKIN HOMING PEPTIDES

This example demonstrates the localization of lung, pancreas and skin homing molecules using immunohistologic examination.

Phage displaying homing peptides were detected in lung, pancreas and skin by immunostaining histologic sections obtained 5 min after administration of phage expressing a lung, pancreas or skin homing peptide ("peptide-phage") to a mouse. Following administration of the peptide-phage, mice were handled as described above and various organs, including lung, pancreas and skin, were removed and fixed in Bouin's solution (Sigma). Histologic sections were prepared and reacted with anti-M13 (phage) antibodies (Pharmacia Biotech; see U.S. Patent No. 5,622,699, *supra*, 1997; Pasqualini and Ruoslahti, *supra*, 1996). Visualization of the bound anti-M13 antibody was performed using a second antibody conjugated to peroxidase (Sigma) according to the manufacturer's instructions.

Phage displaying the lung homing peptide, GFE-1 (SEQ ID NO: 1), were administered intravenously to mice and, after 5 minutes of circulation, the lung was isolated and processed as described above. Immunohistochemical staining of the alveolar capillaries was observed and no preference for any anatomical portion was detected. However, staining of bronchiolar walls and some larger blood vessels was

absent. Mice injected with unselected phage did not exhibit lung staining, and no staining was observed in pancreas and skin after injection of GFE-1 (SEQ ID NO: 1).

Similar experiments were performed in pancreas using phage displaying the pancreas homing peptide, SWCEPGWCR (SEQ ID NO: 4). In these experiments, histological samples of the pancreas as well as control organs and tissues including lung and skin were prepared and examined by immunostaining as described above. The results revealed staining in the capillaries and larger blood vessels of the exocrine pancreas whereas little if any staining of the endocrine pancreas was detected. Again, unselected phage did not stain pancreas, nor was any staining observed in lung and skin of mice injected with phage displaying SWCEPGWCR (SEQ ID NO: 4). Interestingly, some staining of blood vessels within the uterus was observed for the SWCEPGWCR (SEQ ID NO: 4) peptide. Moreover, after intravenous injection of phage displaying SWCEPGWCR (SEQ ID NO: 4), the phage was recovered from uterus at a 6x higher level in comparison to unselected phage. Thus, SWCEPGWCR (SEQ ID NO: 4) homes to both pancreas and uterus.

Experiments were performed in skin using phage displaying the skin homing peptide CVALCREACGEGC (SEQ ID NO: 3). In these experiments, histological samples from the skin as well as control organs and tissues including lung and pancreas were prepared and examined by immunostaining as described above. The results revealed staining in blood vessels of the hypodermis whereas little if any staining of the dermis was detected. Again, unselected phage did not stain

these blood vessels, and no staining was observed in control the lung and pancreas of mice injected with phage displaying CVALCREACGEGC (SEQ ID NO: 3).

All phage, including unselected phage, caused staining of the liver and spleen. This result is consistent with the capture of phage by a component of the RES which was previously described.

These results demonstrate that lung, pancreas and skin homing peptides selectively home to lung, pancreas and skin, particularly to the vasculature. In addition, these results reveal that organs and tissues can exhibit differences of the staining patterns within particular regions, presumably reflecting the differential expression of a target molecule within the organ or tissue. Immunohistochemical analysis provides a convenient assay for identifying the localization and distribution of phage expressing lung, pancreas and skin homing peptides.

Although the invention has been described with reference to the examples provided above, it should be understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the claims.

TABLE 2

PEPTIDES FROM PHAGE RECOVERED FROM LUNG

CIKGNVNC (32)	CRHESSSC (33)
CLYIDRRC (34)	CYSLGADC (35)
CSKLMMTC (35)	CGFELETC* (2)
CNSDVDLC (36)	CVGNLSMC* (37)
CEKKLLYC (38)	CKGQRDFC* (39)
CTFRNASC (40)	CNMGLTRC* (41)
CHEGYLTC* (42)	CGTFGARC (43)
CIGEVEVC* (16)	CRISAHPC (44)
CLRPYLNC* (45)	CSYPKILC (46)
CMELSKQC* (47)	CSEPSGTC (48)
CGNETLRC (49)	CTLSNRFC (50)
CMGSEYWC (51)	CLFSDENC* (52)
CAHQHIQC (53)	CKGQGDWC (54)
CAQNMLCC (55)	CWRGDRKIC* (56)
CLAKENVVC* (13)	CIFREANVC (57)
CRTHGYQGC (58)	CERVVGSSC (59)
CKTNHMESC (60)	CYEEKSQSC (61)
CKDSAMTIC (62)	CTRSTNTGC (63)
CMSWDAVSC* (64)	CKWSRLHSC* (65)
CMSPQRSDC (66)	CLHSPRSKC (67)
CPQDIRRNC (68)	CLYTKEQRC (69)
CQTRNFAQC (70)	CTGHLSTDC (71)
CQDLNIMQC (72)	TRRTNNPLT (73)
CGYIDPNRISQC (74)	CTVNEAYKTRMC* (75)
CRLRSYGTLSLC* (76)	CAGTCATGCNGVC (77)
CADYDLALGLMC (78)	CPKARPAPQYKC (79)
CSSHQGGFQHGC (80)	CQETRTEGRKKC (81)
CRPWHNQAHTEC* (82)	CSFGTHDTEPHC (83)

TABLE 2 (cont.)

CSEAASRMIGVC* (84)	CWEEHPSIKWWC* (85)
CWDADQIFGIKC (86)	CVDSQSMKGLVC (87)
CRLQTMGQGQSC (88)	CRPAQRDAGTSC (89)
CGGRDRGTYGPC (90)	CGEVASNERIQC (91)
CNSKSSAELEKC (92)	CVLNFKNQARDC (93)
CRGKPLANFEDC (94)	CEGHSMRGYGLC (95)
CRDRGDRMKSLC (96)	CDNTCTYGVDDC (97)
CSAHSQEMNVNC (98)	CGAACGVGCRGRC (99)
CGFECVRQCPERC* (1)	CLVGCRLSCGGEC (100)
CRSGCVEGCGGRC (101)	CIARCGGACGRHC (102)
CGGECGWECEVSC (103)	CGVGCPGLCGGAC* (104)
CKWLCLLLCAVAC (105)	CSEGCGPVCWPEC (106)
CGAACGVGCGGRC (107)	CSGSCRRGCGIDC (108)
CGASCALGCRAYC (109)	CDTSCENNCQGPC (110)
CSRQCRGACGQPC (111)	CYWWCDGVCALQC (112)
CAGGCAVRCGGTC (113)	CGGACGGVCTGGC* (114)
CGRPCVGECRMGC (115)	CLVGCEVGCSPAC (116)
CPRTCGAACASPC (117)	CRGDCGIGCRRLC (118)
CCFTNFDCYLGC (435)	

Parentheses contain SEQ ID NO:.

* indicates sequences isolated more than once.

TABLE 3

PEPTIDES FROM PHAGE RECOVERED FROM PANCREAS

EICQLGSCT	(119)	WRCEGFNCQ	(120)
RKCLRPDCG	(121)	SWCEPGWCR*	(4)
LACFVTGCL	(122)	GLCNGATCM*	(123)
DMCWLIGCG	(124)	SGCRTMVCV	(125)
QRCPRSFCL	(126)	LSCAPVICG	(127)
RECTNEICY	(128)	NECLMISCR	(129)
SCVFCDWLS	(130)	WACEELSCF	(131)
QNCPVTRCV	(132)	CATLTNDEC	(133)
CDNREMSC	(134)	CFMDHSNC	(135)
CGEYGREC	(136)	CHMKRDRTC	(137)
CKKRLLNVC	(138)	CLDYHPKC	(139)
CMTGRVTC	(140)	CNKIVRRC	(141)
CPDLLVAC	(142)	CSDTQSIGC	(143)
CSKAYDLAC	(144)	CSKKGPSYC	(145)
CTLKHTAMC	(146)	CTQHIANC	(147)
CTTEIDYC	(148)	CVGRSGELC	(149)

Parentheses contain SEQ ID NO:.

* indicates sequences isolated more than once.

TABLE 4

PEPTIDES FROM PHAGE RECOVERED FROM GUT

YAGFFLV* (150)	RSGARSS (151)
CVESTVA (152)	SRRQPLS* (153)
SKVWLLL (154)	QVRRVPE (155)
YSGKWGW* (156)	MVQSVG (157)
LRAVGRA (158)	MSPQLAT* (159)
GAVLPGE (160)	WIEEAER* (161)
LVSEQLR (162)	RGDRPPY (163)
VRRGSPQ (164)	RVRGPER (165)
GISAVLS* (166)	GGRGSWE (167)
GVSASDW (168)	FRVRGSP (169)
SRLSGGT (170)	WELVARS (171)
MRRDEQR (172)	GCRCWA (173)
LSPPYMW (7)	LCTAMTE (18)

Parentheses contain SEQ ID NO:.

* indicates sequences isolated more than once.

TABLE 5

PEPTIDES FROM PHAGE RECOVERED FROM SKIN

CYADCEGTCGMVC (174)	CWNICPGGCRALC* (175)
GPGCEEECQPAC (176)	CKGTCVLGCSEEC* (177)
CSTLCGLRCMGTC (178)	CMPRCGVNCKWAC (179)
CVGACDLKCTGGC (180)	CVALCREACGEGC* (3)
CSSGCSKNCLEMC* (181)	CGRPCRGGCAASC (182)
CQGGCGVSCPIFC (183)	CAVRCDGSCVPEC* (184)
CGFGCSGSCQMQC (185)	CRVVCADGCRFIC (186)
CTMGCTAGCAFAC (187)	CEGKCGLTCECTC (188)
CNQGCSGSCDVMC (189)	CASGCSESCYVGC (190)
CGGGCQWGCAGEC* (191)	CSVRCKSVCIGLC (192)
CPSNCVALCTSGC (193)	CVEGCSSGCGPGC (194)
CRVVCADGCRLIC (195)	CSTLCGLRCMGTC (196)
CFTFCEYHCQLTC (197)	

Parentheses contain SEQ ID NO:.
* indicates sequences isolated more than once.

TABLE 6

PEPTIDES FROM PHAGE RECOVERED FROM RETINA

CRRIWYAVC (198)	CSAYTTSPC (199)
CSCFRDVCC* (5)	CTDKSWPC (200)
CTDNRVGS (201)	CTIADFPC (202)
CTSDISWWDYKC (203)	CTVDNELC (204)
CVGDCIGSCWMFC (205)	CVKFTYDC[2] (206)
CVSGHLNC (207)	CYGESQQMC (208)
CYTGETWTC (209)	CAVSIPRC (210)
CDCRGDCFC (211)	CDSLCGGACAARC (212)
CERSQSKGVHHC (213)	CFKSTLLC (214)
CFWHNRAC (215)	CGDVCPSECPGWC (216)
CGEFKVGC* (14)	CGLDCLGDCSGAC (217)
CGPGYQAQCSLRC (218)	CGSHCGQLCKSLC (219)
CHMGCVSPCAYVC (220)	CILSYDNPC (221)
CISRPYFC (222)	CKERLEYTRGVC (223)
CKERPSNGLSAC (224)	CKPFRTEC (225)
CKSGCGVACRHMC (226)	CLKPGGQEC (227)
CMDSQSSC* (228)	CMNILSGC (229)
CNIPVTTPIFGC (230)	CNQRTNRESGNC* (231)
CNRKNSNEQRAC (232)	CNRMEMPC (233)
CQIRPIDKC (234)	CAIDIGGAC (235)
CGRFDTAPQRGC (236)	CKRANRLSC (237)
CLLNYTYC* (238)	CLNGLVSMC (239)
CMSLGNNC (240)	CNRNRMTPC (241)
CQASASDHC* (242)	CQLINSSPC (243)
CQRVNSVENASC (244)	CRKEHYPC (245)
CRRHMERC (246)	CSGRPFKYC (247)
CTHLVTLC (248)	CTSSPAYNC (249)
CVTSNLRVC* (250)	CWDSGSHIC (251)
CERSHGRLC[1] (252)	CGNLLTRRC (253)
CINCLSQC (254)	CLRHDFYVC (255)

TABLE 6 (cont.)

CNSRSENC (256)	CRYKGPSC (257)
CSHHDTNC (258)	CSRWYTTC (259)
CYAGSPLC (260)	CQTTSWNC* (261)
CQWSMNVC (262)	CRARIRAEDISC* (263)
CRDVVSVIC (6)	CRREYSAC (264)

Blast-Search:

[1]rat retinal guanylcyclase precursor EC4.6.1.2

[2]rat glutamate receptor subunit epsilon 1 precursor

No stainings for any motif tested, only evidence for preferential homing are the RDV-containig phages in comparison to an ampicillin-phage.

Parentheses contain SEQ ID NO:.

* indicates sequences isolated more than once.

TABLE 7

PEPTIDES FROM PHAGE RECOVERED FROM PROSTATE

EVQSAKW (265)	KRVYVLG (266)
GRLSVQV (267)	WKPASLS (268)
FAVRVVG (269)	LVRPLEG (270)
GFYRMLG (271)	EGRPMVY (272)
GSRSLGA (273)	RVWQGDV (274)
GDELLA (275)	FVWLVGS (276)
GSEPMFR (277)	VSFLEYR (22)
WHQPL (278)	SMSIARL* (21)
RGRWLAL* (279)	QVEEFPC (280)
LWLSGNW (281)	GPMLSVM (282)
WTFLERL (283)	VLPGGQW (284)
REVKES (285)	RTPAAVM (286)
GEWLGEC (287)	PNPLMPL (288)
SLWYLGA (289)	YVGGWEL (290)

Parentheses contain SEQ ID NO:.

* indicates sequences isolated more than once.

TABLE 8

PEPTIDES FROM PHAGE RECOVERED FROM OVARY

EVRSRLS* (10)	RVGLVAR* (11)
AVKDYFR (291)	GVRTSIW (292)
RPVGMRK (293)	RVRLVNL (294)
FFAAVRS (295)	KLVNSSW (296)
LCERVWR (297)	FGSQAFV (298)
WLERPEY (299)	GGDVMWR (300)
VRARLMS (301)	TLRESGP (302)

Parentheses contain SEQ ID NO:.

* indicates sequences isolated more than once.

TABLE 9

PEPTIDES FROM PHAGE RECOVERED FROM LYMPH NODE

WGCKLRFCS (303	MECIKYSCL (304)
GICATVKCS (305)	PRCQLWACT (306)
TTCMSQLCL (307)	SHCPMASLC (308)
GCVRRLLCN (309)	TSCRLFSCA (310)
KYCTPVECL (311)	RGCNGSRCS (312)
MCPQRNCL (313)	PECEGVSCI (314)
AGCSVTVCG* (315)	IPCYWESCR (316)
GSCSMFPCS* (317)	QDCVKRPCV (318)
SECAYRACS* (319)	WSCARPLCG* (320)
SLCGSDGCR (321)	RLCPSSPCT (322)
MRCGFSGCT (323)	RYCYPDGCL (324)
STCGNWTCR (325)	LPCTGASCP (326)
CSCTGQLCR (327)	LECRRWRCD (328)
GLCQIDECR* (329)	TACKVAACH (330)
DRCLDIWCL* (331)	XXXQGSPCL (332)
PLCMATRCA* (333)	RDCSHRSCE* (334)
NPCLRAACI* (335)	PTCAYGWCA* (336)
LECVANLCT* (337)	RKCGEEVCT* (338)
EPCTWNACL* (339)	LVCPGTACV (340)
LYCLDASCL (341)	ERCPMAKCY (342)
LVCQGSPCL (343)	QQCQDPYCL* (344)
DXCXDIWCL (345)	QPCRSMVCA (346)
KTCVGVRV (347)	WSCHEFMCR (348)
LTCWDWSCR (350)	SLCRLSTCS (351)
KTCAGSSCI (352)	VICTGRQCG (353)
NPCFGLLV (354)	SLCTAFNCH (355)
RTCTPSRCM (356)	QSCLWRICI (357)
QYCWSKGCR (358)	LGCFPSWCG (359)

TABLE 9 (cont.)

VTCSSEWCL	(360)	RLCSWGGCA	(361)
STCISVHCS	(362)	EVCLVLSCQ	(363)
IACDGYLCG	(364)	RDCVKNLCR	(365)
XGCYQKRCT	(366)	LGCFXSWCG	(367)
IRCWGGRCS	(368)	IPCSLLGCA	(369)
AGCVQSQCY	(370)	PRCWERVCS	(371)
KACFGADCX	(372)	TLCPLVACE	(373)
SACWLSNCA	(374)	SECYTGSCP	(375)
GLCQEHRCW	(376)	VECGFSAVF	(377)
EDCREWGCR	(378)	HWCRLLACR	(379)

Parentheses contain SEQ ID NO:.

* indicates sequences isolated more than once.

X = Not known.

TABLE 10

PEPTIDES FROM PHAGE RECOVERED FROM ADRENAL GLAND

HKGQVYS (380)	FSDVHFW* (381)
RGIFVSS (382)	PKVKLSE (383)
LRFWQES (384)	IWTVVGQ (385)
DKVGLSV (386)	SETWRQF (387)
LDGMIVK (388)	RYPLAGG (389)
FTDGEDK (390)	RSTEHMS (391)
SGRRHEL (392)	LMLPRAD* (27)
SSSRVRS (393)	YHRSVGR (394)
PLLRPPH (395)	SDKLGFV* (396)
LPRYLLS (28)	AGSRTNR (397)
ITQLHKT (398)	ARCLVYR (399)
GYVAVMT* (400)	GLQVKWV (401)
IFTPGWL (402)	KQTSRFL (403)
R(Y/F)LLAGG (404)	

Parentheses contain SEQ ID NO:.

* indicates sequences isolated more than once.

TABLE 11

PEPTIDES FROM PHAGE RECOVERED FROM LIVER

	ARRGWTL	(405)	SRRFVGG*	(406)
	QLTGGCL	(407)	ALERRSL	(408)
10	KAYFRWR	(409)	RWLAWTV	(410)
	VGSFIYS*	(411)	LSLLGIA	(412)
	LSTVLWF	(413)	SLAMRDS	(414)
	GRSSLAC	(415)	SELLGDA	(416)
	CGGAGAR	(417)	WRQNMPL*	(418)
15	DFLRCRV	(419)	QAGLRCH	(420)
	RALYDAL	(421)	WVSVLGF	(422)
	GMAVSSW	(423)	SWFFLVA	(424)
	WQSVVRV	(425)	VKSVCRT*	(12)
	CGNGHSC	(426)	AEMEGRD	(427)
20	SLRPDNG	(428)	PAMGLIR	(429)

Parentheses contain SEQ ID NO:.

* indicates sequences isolated more than once.